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THE UNIVERSITY OF ALBERTA

THE ROLE OF VAGAL AFFERENTS IN THE REGULATION OF BRONCHIAL
SMOOTH MUSCLE TONE

by

VINEET GOEL

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

IN

PHARMACEUTICAL SCIENCES (PHARMACOLOGY)

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

EDMONTON, ALBERTA

SPRING 1986

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ISBN 0-315-30319-0

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To My Parents

ABSTRACT

The role of vagal C-fiber (pulmonary and bronchial) afferents in causing bronchospasm was studied in anesthetized paralyzed guinea pigs. Two pharmacological approaches were used. Firstly, C-fiber afferents were stimulated by intravenous (i.v.) and intra-aortic (i.a.) injections of selective agonists, phenylbiguanide (PBG), lobeline, nicotine, 5-H.T., and capsaicin. Their effects were compared to those of sodium cyanide (NaCN), a chemoreceptor stimulant, and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), a ganglionic stimulant. Secondly, the effects of electrical stimulation of the vagus were analyzed to characterize the transmitter, if any, at vagal afferent endings. Breath-by-breath measurements of pulmonary-flow resistance (R) and dynamic thoracic elastance (E) were made using computerized techniques and mean arterial blood pressure (MABP) was recorded. A technique was developed to inject drugs into the aortic arch and into the dorsal aorta.

PBG (50-500 $\mu\text{g/kg}$) injected i.v. and i.a. had no effect on R and E, but given i.v. it increased MABP, and, given i.a., a fall followed by an increase in MABP. Bilateral vagotomy and bilateral glossopharyngealotomy, decentralization, or 6-hydroxydopamine (50 mg/kg, i.p., 24 h beforehand) eliminated responses to PBG injected i.v. or i.a. Pressor responses were reduced significantly by atropine, mepyramine, sodium cromoglycate, mecamlamine, and bethanidine. Based on these results it is concluded that PBG induced centrally mediated reflex changes in MABP via vagal and glossopharyngeal afferents and sympathetic efferents. NaCN (50-500 $\mu\text{g/kg}$) injected i.v. or i.a. induced dose-dependent increases in MABP without affecting R and E. Pressor responses to NaCN were reflex — bilateral vagotomy and bilateral glossopharyngealotomy reduced them. Lobeline (0.2-1.0 mg/kg) i.v. or i.a. had insignificant effects on R, E, or MABP. Nicotine (20-200 $\mu\text{g/kg}$) and DMPP (5-40 $\mu\text{g/kg}$) injected i.v. or i.a. had no bronchoconstrictor effects, but induced dose-dependent increases in MABP. Nicotine-induced responses were non-reflex — bilateral vagotomy or mecamlamine was without effect on them. 5-H.T. injected by either route induced dose-dependent increases in R and E, that were eliminated by methysergide. Changes in R and E were non-reflex — bilateral vagotomy, atropine, mecamlamine, pentolinium, trimetaphan, and bethanidine were

without effect.

Capsaicin given i.v. or i.a., induced dose-dependent increases in R and E. Threshold doses were 0.5–1.0 $\mu\text{g/kg}$; $>8.0 \mu\text{g/kg}$ induced tachyphylaxis. These responses to capsaicin, i.v. or i.a., were similar after decentralization, bilateral vagotomy, or glossopharyngealotomy, and after autonomic blockers (mecamylamine, atropine, mepyramine, and bethanidine). Capsaicin (2.0–32.0 $\mu\text{g/kg}$, i.v. or i.a.) had no bronchospastic effect in guinea pigs given this drug (50 mg/kg, s.c.) 7 days earlier, suggesting a role for tachykinins. Antagonists to substance P (SP), [D-Pro²,D-Trp^{7,8}]-SP and [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP, given i.v. significantly reduced capsaicin-induced increases in R but had no effect on increases in E.

I.v. and i.a. injections of SP (2.0–4.0 $\mu\text{g/kg}$), physalaemin (PH) (0.5–1.0 $\mu\text{g/kg}$), and eleodoisin-related peptide (ERP) (0.5–2.0 $\mu\text{g/kg}$) induced dose-dependent increases in R and E and falls in MABP. Increases in R and E due to these tachykinins were non-reflex — bilateral vagotomy, atropine, mepyramine, and mecamylamine had no effect. [D-Pro²,D-Trp^{7,8}]-SP reduced ERP-induced increases in R and E but was without effect on SP- and PH-induced increases in both parameters. [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP significantly reduced SP- and PH-induced increases in R and E, but not those induced by ERP. Thus, [D-Pro²,D-Trp^{7,8}]-SP was more specific at SP-E receptors, and [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP was more effective at SP-P receptors.

Central electrical stimulation of one vagus with the other intact had no bronchospastic effects, but peripheral stimulation of one or both caused increases in R and E that were dependent upon pulse duration and the frequency of the stimuli. Responses to bilateral stimulation were much larger than those to unilateral stimulation. Increases in R and E due to submaximal vagal stimulation were abolished by atropine and mecamylamine, but they only partly blocked increases in R and E due to maximal vagal stimulation. This atropine-resistant bronchoconstrictor component was absent in animals pretreated with capsaicin. [D-Pro²,D-Trp^{7,8}]-SP significantly reduced atropine-resistant increases in R but not those in E. [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP significantly reduced increases in both R and E induced by vagal stimulation in atropine-treated animals. Thus, there are two components to the

bronchospasm induced by vagal stimulation: one due to orthodromic stimulation of vagal *efferents* which is blocked by atropine and mecamlamine, the other due to antidromic stimulation of vagal *afferents* which is selectively abolished by capsaicin pretreatment and which is reduced by antagonists to SP.

The lack of bronchospastic effect of PBG, NaCN, lobeline, nicotine, and DMPP and the absence of any reflex bronchospastic components in airway responses to 5-H.T. and capsaicin suggests that none of the sensory receptors affected by these agonists are involved in centrally mediated reflex bronchospasm in guinea pigs. This suggests that the guinea pig is not a good model to study reflex bronchoconstrictor effects. Capsaicin induces bronchospasm by releasing SP or related tachykinins from vagal afferents. [D-Pro²,D-Trp^{7,8}]-SP is more effective at SP-E receptors whereas [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP is more effective at SP-P receptors. In the large airways, mainly SP-E receptor specific tachykinins are released, but in small airways SP-P receptor-specific tachykinins predominate. Thus, the guinea pig appears to be a good model for studying the role of tachykinins in lung disease.

Acknowledgements

I wish to thank Dr. D.F. Biggs for his supervision, assistance, and encouragement throughout the course of this work. I thank Dr. R.L. Jones and Dr. A.J.D. Friesen, members of the Supervisory Committee, for their constructive criticism of this work from time to time. I also thank Mr. C. Ediss, Faculty Service Officer, for writing the computer program.

Finally, I wish to thank the Faculty for providing financial assistance.

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List of Abbreviations

AMP:	Adenosine monophosphate
ASA:	Acetylsalicylic acid
BDC:	Bottom dead center
B.P.:	Blood pressure
C:	Dynamic thoracic compliance
C _L :	Dynamic pulmonary compliance
COLD:	Chronic obstructive lung disease
d:	Specific gravity
DMPP:	1,1-dimethyl-4-phenylpiperazinium iodide
E:	Dynamic thoracic elastance
E _L :	Dynamic pulmonary elastance
ECF-A:	Eosinophil chemotactic factor of anaphylaxis
ECG:	Electrocardiogram
ERP:	Eledoisin-related peptide
5-HETE:	5-hydroxy-6,8,11,14-eicosatetraenoic acid
HMW-NCF:	High molecular weight neutrophil chemotactic factor of anaphylaxis
i.a.:	Intra-aortic
i.p.:	Intra-peritoneal
i.v.:	Intravenous
Ig:	Immunoglobulin
J:	Juxtapulmonary capillary
LT:	Leukotriene
MABP:	Mean arterial blood pressure
n:	Viscosity
NaCN:	Sodium cyanide
NANC:	Non-adrenergic non-cholinergic

P:	Transthoracic pressure
PAF:	Platelet activating factor
PG:	Prostaglandin
PH:	Physalaemin
r:	Radius
R:	Pulmonary-flow resistance
Re:	Reynold's number
s.c.:	subcutaneous
SCG:	Sodium cromoglycate
SP:	Substance P
SRS-A:	Slow reacting substance of anaphylaxis
TDC:	Top dead centre
TDI:	Toluene di-isocyanate
TX:	Thromboxane
v:	Air velocity
V:	Volume
$\dot{V}$:	Flow rate
VIP:	Vasoactive intestinal polypeptide

1. INTRODUCTION

1.1 Historical Aspects

The word "asthma" is derived from the Greek word for panting. The term was used by Hippocrates (460-370BC), Aretaeus (81-138AD), and Galen (139-199AD) to describe any condition associated with dyspnea (see Sakula, 1984). The first detailed description of asthma by Maimonides (1135-1204) appeared in 1190 (see Rosner, 1984), but it was not until the 17th century that asthma was distinguished clinically from other types of dyspnea (Sakula, 1984). At this time, asthma was considered to be a type of nervous or convulsive "fit" similar to epilepsy. In the first major monograph on asthma, Floyer (1698), defined asthma clearly for the first time and distinguished it from other pulmonary disorders. He considered that the cause of dyspnea was bronchial constriction and regarded the spasm as tonic and more akin to catalepsy than to the clonic convulsions of epilepsy (see Sakula, 1984). Little progress in understanding the disease process was made in next two hundred years.

In 1912, Barker and Sladen noted the bronchoconstrictor and bronchodilator actions of pilocarpine and atropine respectively in asthmatics. Nine years later, Alexander and Paddock (1921) noted the beneficial effect of epinephrine on pilocarpine-induced "asthma-like" attacks, and suggested that the basic abnormality of asthma was imbalance between the cholinergic and the sympathetic nervous systems. Tiffeneau (1957) recommended determining the degree of bronchial hyperreactivity for diagnosis, and for ascertaining the prognosis of asthmatic patients. In 1962, the American Thoracic Society defined asthma as a disease characterized by increased responsiveness of the airways to various stimuli. In the last two decades many papers have been published on various aspects of this "hyperreactivity".

1.2 Epidemiology

The social costs, morbidity, and mortality associated with asthma is high. About 4 per cent of the North American population (up to 15% of children under 15 yr; Esquibel et al., 1984) have asthma, and the effects of the disease are felt by all family members. Most

patients require treatment, many require hospitalization periodically, and majority are forced to limit their activities (see Bonner, 1984).

The prevalence of asthma is highest among children and older adults (Wilder, 1973; Esquibel et al., 1984). In all age groups, asthma is slightly more common in males than females but the ratio differs among age groups. The changing sex ratios may, in part, reflect increased exposure to respiratory infections among women during their child-bearing years, and the influence of smoking and the cumulative effects of exposure to occupational hazards in older men. Asthma is less prevalent among Europeans than among other races, and is uncommon in American Indians and Inuit (Bonner, 1984). The differences in prevalence among the races are not related to socioeconomic status (Mak et al., 1982), but could involve environmental factors, as suggested by studies documenting a relatively low prevalence of asthma in the non-white, developing countries (Cookson & Makoni, 1980). The prevalence of asthma is also influenced by environmental factors such as air pollution, respiratory infections, smoking, occupational exposures, and breast feeding (Bonner, 1984).

In 1978, there were more than 75,000 'asthma-related' (asthma, chronic bronchitis, and emphysema) hospital admissions in Canada, amounting to a total of more than 500,000 hospital days (Hospital Morbidity, 1979-80; 1980-81). In the same year, there were more than 2,500 'asthma-related' deaths (World Health Statistics, 1980) of which more than 300 were labelled as "deaths due to asthma" (Causes of Death, 1983) (Fig. 1). In Canada, rates of death from asthma have remained low and constant for most of the last decade, but have increased in the last 5 years (Fig. 1). Similar increases have been reported in other countries (Benatar, 1986), including the U.K. (Stableforth, 1983; Cushley & Tattersfield, 1983), United States (Sly, 1984), and New Zealand (Jackson et al., 1982; Sly, 1984; Sears et al., 1985). At present it is not known whether the increased mortality from asthma is due to an increase in the prevalence or severity of asthma, or to an increase in case-fatality rates (Sly, 1984).

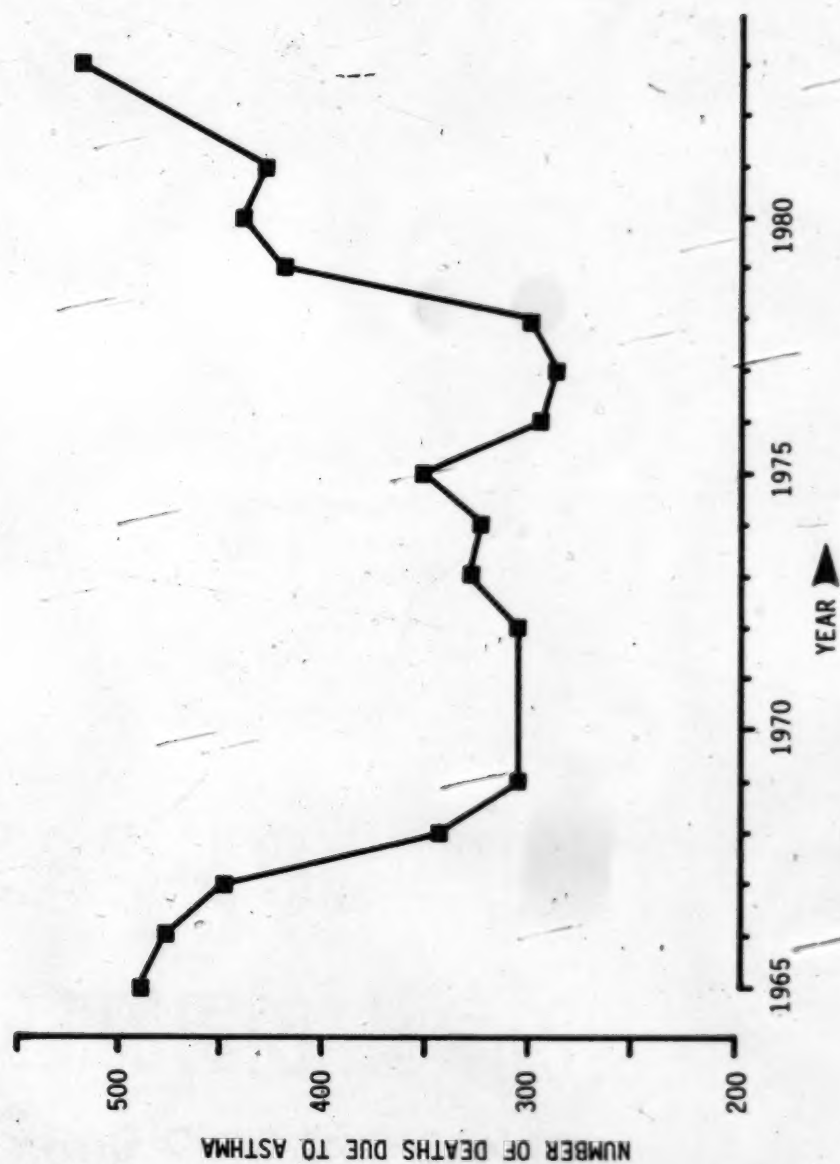


Figure 1. Asthma mortality in Canada (1965-1983).

1.3 Airway Morphology

The airways can be divided into the large or conducting airways and the small distensible or peripheral airways. The large airways include the trachea and the bronchial tree down to the level of terminal bronchioles; the small airways encompass those airways between the terminal bronchioles and the alveoli (Nash, 1976; Gil, 1982). The large airways function as air conduits and the small airways principally participate in respiratory gas exchange.

From the trachea to the respiratory bronchioles, the airways divide by irregular dichotomy, i.e., a mother branch divides into two daughter airways of different diameter which, in turn, become parent branches (Gil, 1982). At the level of the third rib in guinea pigs (Cooper & Schiller, 1975), and at the junction of manubrium with the body of the sternum in man (Nash, 1976), the trachea bifurcates into the right and the left main bronchi. The pattern of bifurcation in relation to the mechanical properties of the lungs is discussed later (see Section 1.9). Each main bronchus divides into the lobar bronchi, one going to each lobe of the lung. In the guinea pig, the right lung has four, and left lung three, lobes (Cooper & Schiller, 1975) as compared to three and two lobes in the human right and left lungs respectively (Nash, 1976; Gil, 1982). Each lobar bronchus bifurcates into small divisions, and in man the average number of generations down to terminal bronchiole is 16 (trachea taken as 0) (Gil, 1982). The terminal bronchioles end in respiratory bronchioles that open into the alveolar ducts. The alveolar ducts communicate with several alveolar sacs, and each sac comprises several alveoli.

The alveoli are thin walled sacs that have one open side, and are connected to adjacent alveoli via pores of Köhn - the intra-alveolar pores about 7-9 μm in diameter (Fig. 2) (Burri, 1985). These pores provide a pathway for collateral ventilation in cases of small airway obstruction and may be involved in the spread of infection from one alveolus to another (Burri, 1985). Inter-alveolar pores are rare or absent in children and increase in number with age (Nash, 1976).

The trachea and bronchi are lined by pseudostratified ciliated columnar epithelium, and the bronchioles by ciliated cuboidal epithelium; a flat surface epithelium is found in the

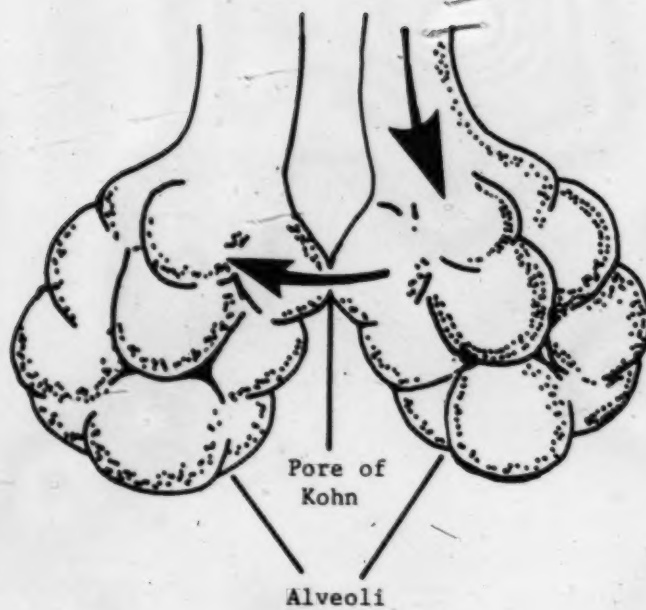


Figure 2. Pores of Kohn.

alveoli (Hogg, 1983). There are at least five distinct types of epithelial cell — ciliated, goblet, basal, Kultschitzky, and Clara cells (see Richardson & Ferguson, 1980).

Ciliated cells form the bulk of the epithelial cells and are responsible for moving mucous secretions. Mucus is synthesized and secreted by the cells of the surface epithelium (goblet cells) and by the submucosal glands. The contribution from these sources depends upon the relative density of goblet cells and submucosal glands in airways. In healthy adult humans, goblet cell volume is 2.5% of the volume of submucosal glands (Reid, 1960); potentially then, the submucosal glands make the greater contribution to the production of respiratory tract mucus. The occurrence and distribution of goblet cells varies among species; generally they are scanty or absent in small animals (Nadel et al., 1985), and the airways of specific pathogen-free (SPF) adult animals contain few goblet cells. By contrast, these cells become more numerous when the airways of SPF animals are exposed to airway irritants (Gil, 1982). Basal cells are 'totipotent' and can differentiate into ciliated or any of the other nonciliated-cell types (Weibel, 1985).

The physiological function of Kultschitzky cells — a type of APUD (Amine content, amine Precursor Uptake, amino acid Decarboxylase) series of cells — in the airways is unknown (Richardson & Ferguson, 1980; Weibel, 1985). Cutz et al., (1975) have suggested that these cells act as sensory receptors, but others (Said & Mutt, 1970; Gil, 1982) consider them to be a source of vasoactive intestinal polypeptide (VIP). The Clara cells in the bronchi and bronchioles secrete the mucopolysaccharides and proteins that form part of the subphase (base layer) of the alveolar surfactant (Nash, 1976).

The epithelium lining the alveoli is composed mostly of Type I (squamous and membranous) and Type II (cuboidal and granular) pneumocytes (Weibel, 1985). Type I cells line most of the alveolar surface and form the major permeability barrier of the lung. Unlike Type I cells, Type II cells are rich in organelles and differentiate to Type I cells. Type II cells secrete the phospholipid layer of the surfactant (Nash, 1976). Recently, Type III or alveolar brush cells have been demonstrated in the alveoli. These cells are structurally similar to the chemoreceptor cells found in taste buds. Their function in lung is unknown (Weibel, 1985).

Epithelial cells in the airways are joined by several types of junctions — tight junctions, desmosomes, nexus or gap junctions, and orthogonal arrays or rectilinear entities (see Richardson & Ferguson, 1980). All these types of junctions have been described in the epithelium of guinea-pig trachea, but their frequency varies among animals (Inoue & Hogg, 1977). The functional significance of these junctions, apart from being a permeability barrier, is unclear. Nexus junctions are believed to be important for coordinating ciliary movements.

The epithelial lining along with its fluid layer (mucous or surfactant), forms a protective barrier against foreign material. The junctions between the epithelial cells form an important component of the protective barrier (Hogg, 1983). The tight junctions can be rendered permeable to tracer proteins by ether, cigarette smoke, cholinergic agonists, and histamine (see Richardson & Ferguson, 1980). These changes in epithelial permeability could contribute to the production of hyperreactivity.

Finally, there are intraepithelial afferent axons (Laitinen et al., 1985). In mouse, abundant C-fiber endings have been found associated with alveolar Type I pneumocytes. They appear to be the equivalent of Paintal's J receptors (Hung et al., 1973). Detailed morphological mapping of these receptors has not been done in human or guinea-pig lungs.

The airway submucosa contains seromucous glands that are the main source of tracheobronchial secretions. In some species, e.g., goose, rat, and rabbit, the submucosal glands are scarce or absent; therefore, in these species the surface epithelial cells are the primary source of mucus (Nadel et al., 1985). The secretions are emptied onto a mucociliary blanket via a glandular duct system. The quality of secretions is influenced by autonomic nervous activity (see Section 1.6).

The outer layers of the trachea and the bronchi are made up of fibrocartilagenous layers that contain the airway smooth muscle and the cartilage. The trachea contains U-shaped cartilage rings connected dorsally at the tip by smooth muscle. In the bronchi, U-shaped cartilage rings are absent, but irregular cartilagenous plates connected by bundles of smooth muscle cells are found. Cartilage is absent in the walls of the bronchioles. The smooth muscle extends from the trachea all the way down to the alveolar ducts (Nadel, 1965; Olsen

et al., 1967). In the bronchi, the muscle is arranged in a geodesic pattern such that, when the muscle contracts, the airway shortens and narrows (Gil, 1982).

1.4 Cells of The Pulmonary Defense System

The lung is exposed to many potential hazards because it has a large surface exposed to the outside air. Therefore, it has an elaborate defense system. The lungs contain cells that act both via the immune system and via direct nonimmune mechanisms.

Nonimmune defense is carried out by macrophages that phagocytose noxious materials and digest them via lysosomal systems. However, not all materials ingested by macrophages can be digested by lysosomal enzymes. Macrophages sequester inorganic dust particles, carbon, asbestos, and silica, but do not modify these materials. These phagocytic cells are found throughout the lung, and are abundant in the surface lining of the alveoli, in the mucous layer of the conducting airways, and in the interstitial space, particularly in the perivascular and peribronchial sheaths, subpleural tissues, and interalveolar septa (Weibel, 1985). Macrophages are derived from monocytes, which in turn originate from 'pluripotent' stem cells in the bone marrow (Furth, 1985). Alveolar macrophages are a major source of platelet-activating factor (PAF), a membrane-derived mediator (Hamasaki et al., 1984).

The cells of the immune defense system comprise lymphocytes and plasma cells. Based on their function, two classes of lymphocytes are differentiated (for review see Green et al., 1977). B cells generate immunoglobulins (Ig), thus, they are related to plasma cells and provide humoral defense through antibodies. T cells exert their defense function through their cell membrane, which carries receptors allowing T lymphocytes to bind to cells bearing certain antigens. T cells are the principal mediators of graft rejection. The immune defense cells are concentrated along the conducting air passages where most noxious intruders (dust particles and airborne bacteria) are intercepted, and are therefore termed bronchus-associated lymphoid tissue (BALT) (Furth, 1985; Weibel, 1985).

Granulocytes, particularly eosinophils and neutrophils, are found in small numbers in places where other free cells occur, particularly in the walls of the bronchi. These cells migrate

into the tissues and traverse the endothelium under the influence of a number of chemotactic substances, e.g., eosinophil chemotactic factor of anaphylaxis (ECF-A), high molecular weight neutrophil chemotactic factor (HMW-NCF), leukotriene (LT) B₄, and 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) (Holgate, 1983). Neutrophils, which accumulate during acute inflammation, damage lung tissue by secreting a variety of hydrolytic enzymes. However, eosinophils commonly accumulate in allergic diseases.

Mast cells are believed to be the primary source of mediators though basophils also contribute (Holgate et al., 1986). Mast cells are found in large numbers throughout the respiratory passages and are most abundant in the conducting airways towards the periphery of the lung (Holgate et al., 1985). In human bronchi, mast cells are located predominantly between the epithelium and the basement membrane, though some can be identified between the epithelial cells, and underneath the basement membrane (Fig. 3) (Jeffrey & Corrin, 1984). From studies in rodents, mast cells have been divided into two groups — mucosal mast cells and connective tissue mast cells — differing in their morphological, histochemical, biochemical, and pharmacological characteristics (Metcalf, 1983; Shanahan et al., 1984). Mucosal mast cells convert arachidonic acid mainly via the lipoxygenase pathway to LTC₄ and LTD₄, whereas connective tissue mast cells produce mainly prostaglandin (PG) D₂ via the cyclooxygenase pathway (Holgate et al., 1985). Mast-cell heterogeneity has not been established in human lung tissue (Holgate et al., 1986). Basophils are generally regarded as being functionally similar to mast cells and are thought to be involved in acute allergic responses.

1.5 Vascular Supply to the Lungs

The lungs receive blood from two different vascular systems, the pulmonary and bronchial circulations. The large airways are supplied by bronchial vessels which emerge from the right intercostal, the right subclavian, or the right internal mammary artery, and directly from the upper thoracic aorta on the left side (Murray, 1976); the small or peripheral airways receive blood from the pulmonary circulation. The pulmonary circulatory flow consists of

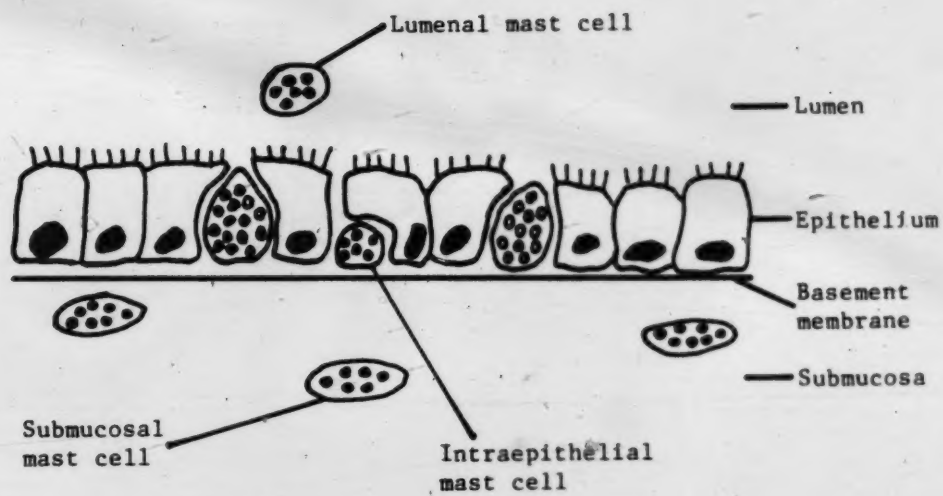


Figure 3. The distribution of mast cells in human bronchi.

virtually the entire output from the right side of the heart. By contrast, blood flow through bronchial circulation is less than 1-2% of the output from the left side of the heart (Murray, 1976). Injection of drugs into the pulmonary circulation (i.e., i.v. injection), tends to localize effects on the small airways initially. Similarly, injection into the bronchial arteries (e.g., intra-aortic injection) produces changes on the large airways initially (Nadel, 1965; Dekock et al., 1966; Dautrebande, 1970). In practice, such demarcations are less than clear-cut due to the presence of 'anastomoses' between the pulmonary and bronchial circulation (Fig. 4). Anastomoses are common in guinea-pig lungs (Willis, 1919/20; Verloop, 1949; Rakshit, 1949/50). In man, anastomoses are sparse in normal lungs, more frequent in infants, and infants with certain congenital disorders, e.g., Fallot's Tetralogy, and in some disease states, e.g., emphysema (Murray, 1976).

1.6 Innervation of the Lungs

The nerve supply to the lungs consists of both sensory (afferent) and motor (efferent) pathways.

1.6.1 Afferent Innervation

This subject has been much reviewed (Paintal, 1973, 1977; Widdicombe, 1981; Sant'Ambrogio, 1982; Coleridge & Coleridge, 1984). Briefly, the lungs have afferent inputs via the spinal cord (sympathetic) and the medulla (vagal).

1.6.1.1 Sympathetic Afferents

Both myelinated and unmyelinated spinal afferents travel in sympathetic nerve branches and are therefore termed 'sympathetic' afferents (Kostreva et al., 1975). The role of these unmyelinated afferents is unknown. Stimulation of myelinated afferents by irritant chemicals induces disturbances in the pattern of breathing reflexly (Widdicombe, 1954; Coleridge et al., 1983).

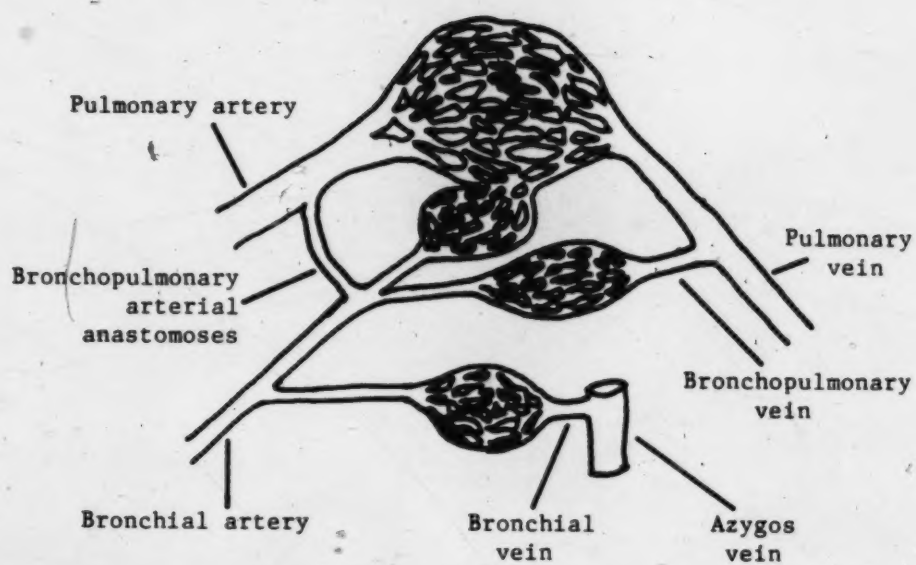


Figure 4. Schematic representation of broncho-pulmonary anastomoses

1.6.1.2 Vagal Afferents



The medullary afferents arise from pulmonary sensory receptors and travel in the vagus. In cats, studies of the degeneration of the vagus nerve and its branches have shown, that 80% of the vagal afferents are unmyelinated (Agostoni et al., 1957). There are four types of vagal afferent end organs in the lungs: slowly adapting pulmonary stretch receptors, rapidly adapting irritant receptors, pulmonary C-fibers or J receptors, and bronchial C-fibers (Paintal, 1973; Coleridge & Coleridge, 1984; Widdicombe, 1985). In cats, dogs, and rabbits, these receptors and their reflex effects (Table 1) can be differentiated neurophysiologically.

Pulmonary stretch receptors are the best known receptors of the lungs. These receptors have myelinated afferents lying in close association with the smooth muscle of large airways (Fig. 5) (Sant'Ambrogio, 1982; Pack et al., 1984). No role for these receptors in lung disease has been demonstrated (Widdicombe, 1985). Stretch receptors help mediate the Hering-Breuer-inflation and deflation reflex, and Head's paradoxical reflex. Stretch receptors also reflexly mediate bronchodilation, positive chronotropic effects, and falls in peripheral vascular resistance (see Paintal, 1977). Recently, they have been shown to be involved in the cough reflex (Sant'Ambrogio et al., 1984).

Pulmonary-irritant receptors are located in the large airways (as far as the proximal end of the respiratory bronchioles) and are most numerous at the carina (Fig. 5). The afferents from these receptors are myelinated, but their terminals, which are situated in airway epithelium and submucosa (Sant'Ambrogio, 1982), are unmyelinated (Laitinen et al., 1985). The most superficial endings lie less than 1 μ m from the airway lumen where they are well sited to respond to intraluminal irritation (Das et al., 1978). These afferent endings initiate the cough reflex, and the ease with which this reflex can be evoked is directly proportional to the number of irritant receptors in the region stimulated (Das et al., 1978). Endings of this type are absent in the mouse and ferret, and this explains why the cough reflex is absent in these species (Pack et al., 1981). Irritant receptors, respond to a variety of stimuli — gaseous irritants, mechanical

Table 1: Characteristics of Pulmonary Vagal Sensory Receptors

Receptor	Location	Fiber type	Natural stimulus	Reflex responses	References
Stretch or slowly adapting receptors	Associated with smooth muscle of bronchi & bronchioles	Myelinated	Lung inflation, increased transpulmonary pressure	Hering Breuer reflex, decreased peripheral vascular resistance, bronchodilation, tachycardia	Paintal, 1973; Widdicombe, 1981; Paintal, 1981
Irritant or rapidly adapting receptors	Epithelium of (mainly) extrapulmonary airways	Myelinated	Irritants, pneumothorax, rapid & deep inflation and deflation, mechanical stimulation, pulmonary congestion, anaphylaxis	Bronchoconstriction, hyperpnoea, cough	Paintal, 1973; Nadel, 1980a; Widdicombe, 1981, 1985
Type 'J' receptors	Alveolar wall	Unmyelinated	Pulmonary congestion, pulmonary edema, microembolism	Tachypnoea, bronchoconstriction, hypotension, bradycardia, J-reflex	Paintal, 1969, 1970, 1973; Anand & Paintal, 1980
Bronchial C-fibers	Epithelium of bronchi & bronchioles	Unmyelinated	Irritants	Bronchoconstriction, hypotension, bradycardia	Coleridge & Coleridge, 1977, 1984, 1985

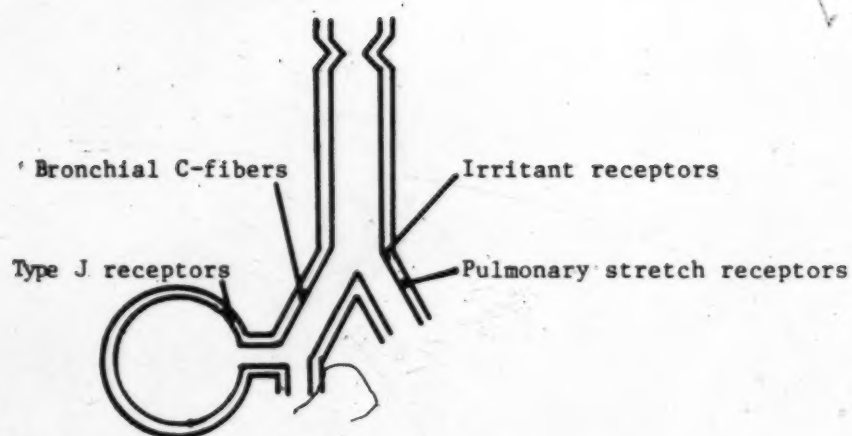


Figure 5. Sensory receptors in the lungs.

stimulation of the larynx and airways, pneumothorax, hyperpnoea, pulmonary arterial microembolism, anaphylactic reactions, and to histamine (Paintal, 1977). Stimulation of irritant receptors also evokes reflex bronchoconstriction (Nadel, 1980a, 1980b).

C-fiber receptors are found throughout the tracheobronchial tree and alveoli, and are usually divided into the 'pulmonary' and 'bronchial' groups depending whether these endings are accessible through the pulmonary or the bronchial circulations when chemicals are injected into the blood stream (Fig. 5) (Sant'Ambrogio, 1982; Coleridge & Coleridge, 1984). Pulmonary C-fibers are also called juxtapulmonary capillary receptors or J receptors as they are located very close to the pulmonary capillaries (Fig. 6) (Paintal, 1973). Their major physiologic stimulus is pulmonary congestion, i.e., an increase in pulmonary capillary pressure (Paintal, 1970), but they are also activated by other stimuli, e.g., deflation and hyperinflation of unphysiologic magnitude, increases in left atrial pressure, acute pulmonary edema, multiple pulmonary emboli, and alveolar inflammation, and during exercise (especially) at high altitudes (see Paintal, 1977). J-receptor stimulation causes visceral and somatic reflex effects. The primary triad of visceral responses comprises bradycardia, hypotension, and apnea followed by tachypnea (Paintal, 1973). Stimulation of C-fiber afferents also induces reflex bronchoconstriction; evidence for this is most convincing in cats (Paintal, 1969), rabbits (Karczewski & Widdicombe, 1969b), and dogs (Russell & Lai-Fook, 1979), but it is lacking in guinea pigs. The main somatic reflex effect produced by type J receptors is the J reflex — inhibition of reflexly induced somatic muscle activity. Paintal (1970) suggests that this reflex operates during severe muscular exercise, following the series of events shown (Fig. 7) (from Paintal, 1970).

Bronchial C-fiber endings are located in the conducting airways and are sensitive to many of the experimental interventions that excite J receptors. In addition, they are stimulated by lung autocoids, e.g., histamine or bradykinin, that fail to stimulate J receptors. Bronchial C-fibers do not have appreciable reflex effects on peripheral vascular resistance, but, like J receptors, mediate other reflex effects including

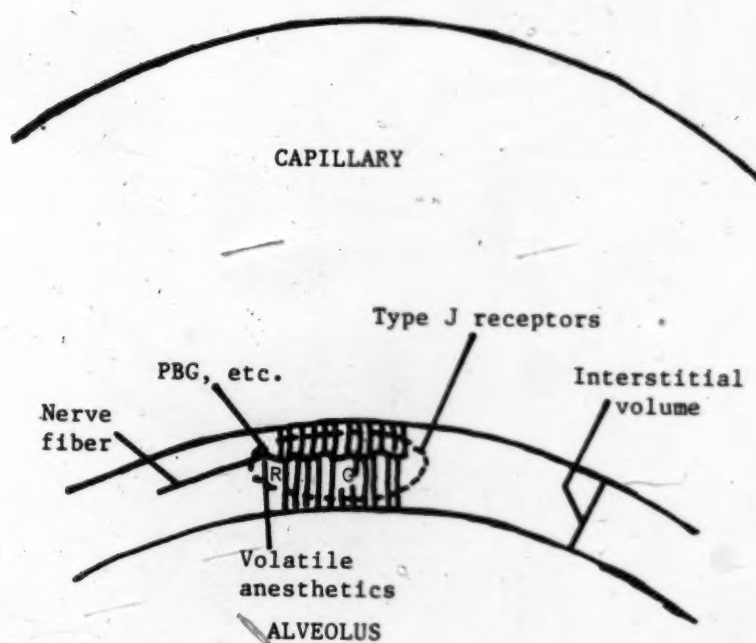


Figure 6. Schematic diagram of the location of J receptors.

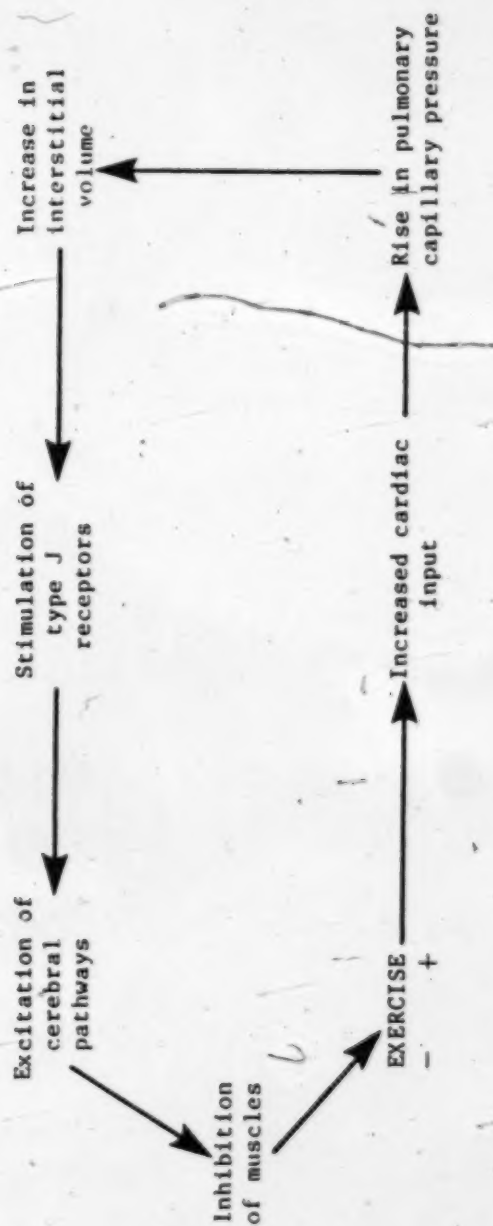


Figure 7. Sequence of events in the J reflex.

bronchoconstriction (Coleridge & Coleridge, 1984; 1985).

1.6.2 Cardiovascular Receptors

In addition to pulmonary receptors, other receptors located in the heart and systemic circulation serve important regulatory functions (Table 2).

1.6.2.1 Arterial Chemoreceptors

Arterial chemoreceptors are located mainly at the bifurcation of the common carotid arteries (carotid bodies), the ascending aorta, the aortic arch, and the origin of the subclavian arteries (Coleridge et al., 1970). They have myelinated or unmyelinated afferent fibers (see Paintal, 1964, 1971; Eyzaguirre & Zapata, 1984). The natural stimulus of both types is hypoxia — the lower the local tissue $[O_2]$ at the receptors, the greater the degree of stimulation (Paintal, 1977). Their afferents are also stimulated by a number of chemical agents depending upon whether they are myelinated or unmyelinated. Carotid-body chemoreceptors are selectively stimulated by CN^- (McQueen, 1980). Afferents from the aortic arch region are carried in the aortic depressor nerve which runs separate from the vagus in rabbits (see Kirchheim, 1976), and guinea pigs (Biggs, personal communication). In guinea pigs, the aortic nerves join the superior laryngeal nerves and these in turn join the vagi at the nodose ganglia. Afferents from the carotid-body and carotid-sinus regions are carried in the carotid-sinus nerve and join the glossopharyngeal (IX) nerve at the jugular foramen.

1.6.2.2 Arterial Baroreceptors

Arterial baroreceptors are found: 1) in the aortic arch at the origin of brachiocephalic artery and the left subclavian artery; 2) in the brachiocephalic artery at its bifurcation into right subclavian and right common carotid artery; and 3) in the carotid sinus region (see Kirchheim, 1976). Afferents from 1) and 2) are carried in the aortic depressor nerve; afferents from the carotid sinus are carried in the carotid-sinus branch of the glossopharyngeal nerve as described above. These receptors can have either

Table 2: Related Cardiovascular Afferent Receptors

Receptor	Location	Fiber type	Natural stimulus	Reflex effects	References
Aortic receptors					
Baroreceptors	Aortic arch & subclavian bifurcation	Myelinated	Hypertension	Bradycardia, hypotension	Kirchheim, 1976
Chemoreceptors	Aortic arch	Myelinated & unmyelinated	Hypoxia, fall in arterial pO_2	Vasoconstriction, hyperpnoea	Biscoe, 1971; Paintal, 1971; 1977
Cardiac receptors					
Type A atrial receptors	Right and left atria at the junction of veins	Myelinated	Increase in atrial wall tension	Unknown	Hakemaki, 1970; Paintal, 1972
Type B atrial receptors	-same-	Myelinated	Increase in atrial volume	Tachycardia, ? bradycardia, diuresis	Coleridge et al., 1957; Paintal, 1972
Atrial receptors with unmyelinated fibers	Right and left atria	Unmyelinated	Unknown	Unknown	Thorén, 1976; Kappagoda et al., 1977
Ventricular pressure receptors	Right and left ventricular walls	Myelinated	Increase in ventricular pressure	Left: bradycardia, hypotension; Right: unknown	Paintal, 1955; Coleridge et al., 1964
Epicaudal receptors	Ventricular surface and in walls	Unmyelinated	Unknown	Bradycardia, hypotension, gastric relaxation	Paintal, 1973; 1977
Pericardial receptors	Pericardium	Myelinated	Cardiac tamponade	Uncertain	Sleight & Widdicombe, 1965; Paintal, 1973; 1977
Carotid receptors					
Baroreceptors	Carotid sinus	myelinated & unmyelinated	Hypertension	Bradycardia, hypotension, bronchospasm	Kirchheim, 1976; Biggs & Peterson, 1981
Chemoreceptors	Carotid body	Myelinated, some unmyelinated	Hypoxia, fall in arterial pO_2	Vasoconstriction, hyperpnoea	Coleridge et al., 1970; Eyzaguirre & Zapata, 1984

myelinated or unmyelinated afferents. Those with myelinated fibers are located in the aortic and carotid regions, but those with unmyelinated fibers have been reported only in the carotid-sinus region (Sato et al., 1968; Fidone & Sato, 1969; Aars, 1971). Baroreceptors are slowly adapting mechanoreceptors (Paintal, 1972) and respond to circumferential and longitudinal stretch induced by changes in transmural pressure (Heymans & Neil, 1958). Afferent discharges from these receptors are increased by the local application of constrictor drugs to the sinus wall and by the electrical stimulation of cervical sympathetic fibers (Koizumi & Sato, 1967; Sampson & Mills, 1970).

Baroreceptor stimulation reflexly reduces efferent sympathetic discharge, thereby inducing hypotension (see Kircheim, 1976). In addition, carotid-sinus afferents in guinea pigs contribute to the bronchospasm induced by a number of drugs, e.g., acetylsalicylic acid (ASA), tartrazine, diazoxide, and 5-H.T. (Biggs & Peterson, 1980; Biggs et al., 1981; Biggs, 1984), and electrical stimulation of carotid-sinus afferents results in reflex bronchoconstriction in this species (Biggs & Peterson, 1981). This bronchoconstrictor response is mainly confined to large conducting airways, involves H_1 - and muscarinic-receptors, and is mediated via the sympathetic division of the autonomic nervous system — vagal afferents or efferents are not involved (see Biggs, 1984). In dogs, Houssay & Orias (1934a, 1934b) reported that carotid-sinus-nerve-stimulation induced bronchoconstriction. By contrast, Daly & Schweitzer (1951) and Nadel & Widdicombe (1962) observed reflex bronchodilation when afferent nervous activity was increased in the carotid-sinus nerve. These latter findings have been confirmed recently (Schultz et al., 1985). One of the most widely used methods of studying the carotid-sinus-baroreceptor reflex involves bilateral occlusion of the common carotid arteries (see Kircheim, 1976).

1.6.2.3 Cardiac Receptors

Most afferent fibers emerging from the atria and ventricles are fine and myelinated (see Paintal, 1981), but unmyelinated endings have been reported in the ventricles (Kulaev, 1963; Coleridge et al., 1964; Sleight & Widdicombe, 1965; Oberg &

Thorén, 1972), and in the right and left atria (Thorén, 1976; Kappagoda et al., 1977). The natural stimulus of these unmyelinated afferents is unknown. Most of the afferents from cardiac receptors are carried in the vagus, though some run in the sympathetic nerves (Nishi et al., 1974; Uchida & Murao, 1974; Lombardi et al., 1976).

1.6.3 Efferent Innervation

The airways are innervated by the sympathetic, parasympathetic and by non-adrenergic non-cholinergic (NANC) nervous systems. Several reviews have appeared in the last 10 years describing the structure and function of these nervous pathways (Nadel, 1977, 1980a; Richardson, 1979; Barnes, 1984c; Nadel & Barnes, 1984; Boushey, 1985; Widdicombe, 1985). Most motor nerves in the airways are not located within the smooth muscle layers, but are arranged in bundles in the submucosa adjacent to the smooth muscle (Pack et al., 1984). These bundles travel to peripheral sites and act by releasing mediators that diffuse into the smooth muscle (Widdicombe, 1985).

1.6.3.1 Sympathetic Efferents

Adrenergic control involves sympathetic nerves releasing norepinephrine in the vicinity of the target organ, and the adrenal medulla secreting catecholamines (mainly epinephrine) into the circulation. Adrenergic control is mediated via bronchodilator β -adrenoceptors, and by bronchoconstrictor α -adrenoceptors.

The sympathetic supply to the lungs is via the upper six thoracic ganglia with long postganglionic fibers (Nadel, 1980a). In humans, the submucosal glands, bronchial vessels, and airway ganglia receive adrenergic innervation, but in airway smooth muscle it is sparse or absent (Richardson, 1979; Nadel & Barnes, 1984; Boushey, 1985). Functional studies have confirmed that bronchial smooth muscle lacks significant sympathetic innervation. Electrical field stimulation of sympathetic nerves causes insignificant effects on human airway-smooth-muscle preparations (Davis et al., 1982). Like humans, guinea-pigs' airways also have sparse sympathetic innervation — adrenergic fibers are present in the trachea, sparse in the bronchi and absent from the bronchioles and small

airways, but densely innervate the pulmonary and bronchial vessels (O'Donnell & Saar, 1973; Foster & O'Donnell, 1975; O'Donnell et al., 1978; McGowan & Niewoehner, 1980).

Sympathetic fibers have been shown to innervate parasympathetic ganglia in the peribronchial plexuses in human airways (Partanen et al., 1982). Thus, it is possible that sympathetic nerves modulate cholinergic neurotransmission at the ganglionic level (Nadel & Barnes, 1984). This might explain why vagal bronchomotor tone has to be present to demonstrate the effect of sympathetic nerve stimulation (Nadel, 1980a). The possibility that sympathetic nerves modulate cholinergic neurotransmission is supported by the finding that norepinephrine inhibits the activity of airway-ganglion cells in cats and ferrets (Baker et al., 1982, 1983). However, Martin & Collier, (1985) reported that norepinephrine did not alter the amount of acetylcholine released from isolated canine tracheal smooth muscle in response to field stimulation.

Pulmonary adrenoceptors have been reviewed recently (Svedmyr, 1983; Barnes, 1984a, 1984b, 1985; Barnes et al., 1984; Nadel & Barnes, 1984). The distribution and the role of these receptors is shown in Table 3. The density of β -receptors is greater than that of α -receptors (Barnes, 1984b). In dogs, the majority (about 80%) are β_2 -receptors, that respond only to exogenous agonists (e.g., epinephrine from adrenal medulla), whereas β_1 -receptors respond to sympathetic nerve stimulation (Barnes et al., 1983b). Therefore, human airway smooth muscle, which lacks sympathetic innervation has few β_1 -receptors. β_2 -Agonists induce bronchodilation by directly relaxing (via cyclic AMP) airway smooth muscle, and also by inhibiting the release of mediators from mast cells. In addition, β_2 -agonists reduce excitatory cholinergic neurotransmission, reduce mucosal edema, and increase mucociliary clearance (Barnes et al., 1984). All these effects could help alleviate the symptoms of asthma.

α -Adrenoceptors that induce airway smooth muscle to contract have been demonstrated in many species, including man (Simonsson et al., 1972; Kneussl & Richardson, 1978). Both α_1 - and α_2 -receptor subtypes are present in airway smooth muscle (Leff & Munoz, 1981), but their ratio varies greatly among species. In canine

Table 3: Efferent Autonomic Pulmonary Receptors.

Receptors	Large Airways	Small Airways	Pulmonary & Bronchial vessels	Submucosal Glands	Mast Cells	Responses	References
Adrenergic							
β_1	+	0	++	?	0	Vasodilation	Nadel & Barnes, 1984
β_2	+	+++	+	++	++	Bronchodilation Mucous secretion Inhibition of mediator release	Barnes et al., 1982 Barnes et al., 1983 Barnes & Basbaum, 1983
α_1/α_2	+	+	+++	++	++	Bronchoconstriction Vasoconstriction Serous secretion Mediator release	Kaliner et al., 1972 Simonsson et al., 1972 Kneussl & Richardson, 1978
Cholinergic							
Muscarinic	+++	+	0	++	?	Bronchoconstriction Mucous secretion Mediator release	Kaliner et al., 1972 Barnes et al., 1983 Nadel & Barnes, 1984 Nadel et al., 1985

0 absent, + present but few, ++ present, +++ present in large numbers, ? uncertain

tracheal smooth muscle, α -adrenoceptor contractile responses are mediated mainly by α_1 -receptors (Barnes et al., 1983b). α -Agonists facilitate allergen-induced release of mediators from human lung fragments (Kaliner et al., 1972), but whether functional α -receptors exist on pulmonary mast cells is unknown (see Nadel & Barnes, 1984). The number of α -receptors in the peripheral lung tissue has been shown to be greatly increased in a guinea-pig model of extrinsic asthma (Barnes et al., 1980). This enhanced α -adrenergic responsiveness is not confined to the airways, as increased pupillary and cutaneous vascular responses to α -agonists are seen in asthmatics (Henderson et al., 1979).

1.6.3.2 Parasympathetic Efferents

Parasympathetic (cholinergic) control involves vagal fibers that release acetylcholine at airway ganglia and (via post-ganglionic fibers) in the vicinity of airway smooth muscle. Effects are mediated via nicotinic receptors in airway ganglia, and muscarinic receptors in the smooth muscle (Table 3).

By contrast to the adrenergic innervation, the parasympathetic innervation is dense in airway smooth muscle, and sparse or absent in the pulmonary vasculature, in most species studied. The nerves enter the lung via the vagus (X) nerves and synapse in ganglia in the airway wall (periarterial and peribronchial plexuses) from these, short postganglionic fibers pass to the smooth muscle and submucosal glands (Richardson, 1979). The peribronchial plexus divides further into the extrachondrial and subchondrial plexuses (named for their relationship to the cartilagenous plates) (Larsell & Dow, 1933; Richardson, 1979). The peribronchial plexus contains myelinated and unmyelinated fibers, but the periarterial plexus contains only unmyelinated fibers (Larsell & Dow, 1933).

In most species, electrical stimulation of vagus nerves innervating the airways releases acetylcholine causing bronchospasm mediated ultimately via muscarinic receptors (Nadel, 1977, 1980a, 1980b; Lundberg et al., 1983a, 1983c; Fryer & MacLagan, 1984; Martling et al., 1984; Blaber et al., 1985). Bronchospastic responses are potentiated by cholinesterase inhibitors (Colebatch & Halmagyi, 1963; Olsen et al., 1965), and are partly

(Lundberg et al., 1983a, 1983c; Martling et al., 1984) or completely (Nadel, 1980a; Fryer & MacLagan, 1984; Blaber et al., 1985) blocked by atropine, depending upon the strength of the stimulus applied. The relative contribution of the conducting and distensible airways to this bronchospasm varies among animals and species (Colebatch & Halmagyi, 1963; Woolcock et al., 1969; Jackson, et al., 1981), and this probably reflects the relative density of the cholinergic innervation to the airways (Nadel & Barnes, 1984) (Table 3). In animals, the secretion from the mucous glands in the airways are increased when the vagal nerves are stimulated directly, or reflexly via mechanical stimulation of the afferents in the gastric mucosa or larynx (Nadel et al., 1985). Also, cholinergic agonists enhance allergen-induced mediator release from human lung fragments (Kaliner et al., 1972), but neither cholinergic effects nor cholinergic receptors have been demonstrated in purified mature mastocytoma cells (see Nadel & Barnes, 1984). There is little or no crossover of vagal motor fibers to the contralateral lung in the thorax (Olsen et al., 1965), but whether central (medullary) crossover occurs has not been investigated.

In most species, resting tone of the airways is maintained via vagal efferent nervous activity (Widdicombe, 1966). The degree of vagal tone differs among species and is influenced by the level and type of anesthesia (Nadel & Widdicombe, 1962; Olsen et al., 1965; Karczewski & Widdicombe, 1969a). In animals, the presence of vagal tone can be demonstrated by cutting or cooling the vagus nerves, or by the administration of atropine. In humans, vagal tone can be demonstrated by administering atropine (Severinghaus & Stupfel, 1955; Nadel & Widdicombe, 1962, 1963; Olsen et al., 1965; Karczewski & Widdicombe, 1969a). Such tone could be of central or peripheral origin; the latter possibly mediated by continuous afferent nervous activity. In cats, Jammes & Mei, (1979) showed that cholinergic tone was abolished by selectively sectioning vagal afferents. They suggested that it originated in pulmonary receptors, and, in particular, peripheral C-fiber afferents.

1.6.4 Non-adrenergic Non-cholinergic Innervation

This comprises non-adrenergic inhibitory (VIP-ergic) nerves and non-cholinergic excitatory nerves.

1.6.4.1 Non-adrenergic Inhibitory Nerves

This system was first demonstrated in 1971 (see Barnes, 1984c), and has received increasing attention in the last 5 years (see Richardson, 1981; Barnes, 1984c). These nerves enter the lungs, probably with the cholinergic nerves, in the vagi. In the absence of significant sympathetic innervation this is the only neurally mediated means of relaxation of human airways (Barnes, 1984c, 1985; Boushey, 1985). Similar nerves are present from the trachea to the bronchioles in guinea pigs (Coburn & Tomita, 1973; see Doidge & Satchell, 1982). A similar inhibitory system has been demonstrated in the pulmonary vasculature in some species (Hamasaki & Said, 1981; Said, 1982). The inhibitory transmitter of this system has not been definitely identified; it seems not to be a purine nucleotide (Irvin et al., 1980; Cameron et al., 1983) and most recent reports suggest that it may be VIP — hence the term peptidergic. VIP has been identified in airway neurons and in bronchial and pulmonary vessels (Uddman & Sundler, 1979; Dey et al., 1981; Polak & Bloom, 1982). Exogenously applied VIP has many of the physiologic and pharmacologic effects of stimulation of this inhibitory system (see Barnes, 1984c). In addition, VIP is released in response to field stimulation of tracheobronchial tissue and induction of tachyphylaxis to VIP reduced responses to stimulation of these inhibitory nerves (Ito & Takeda, 1982). VIP's weak effects in some studies of human airway tissue (see Barnes, 1984c; Palmer et al., 1985), and other evidence (e.g., Karlsson & Persson, 1983), suggest that VIP is probably not the only peptidergic inhibitory neurotransmitter in the airways. This cannot be proved (or disproved) until specific antagonists are found.

1.6.4.2 Non-cholinergic Excitatory Nerves (non-VIP)

In guinea-pig lungs, non-cholinergic excitatory nerves have been demonstrated by electrically stimulating the vagi after cholinergic blockade with atropine (Lundberg et al.,

1983a, 1983c; Martling et al., 1984). Such nervous stimulation induces bronchospasm and increases mucosal permeability, and both these responses are abolished by [D-Arg¹, D-Pro², D-Trp^{7,8}, Leu¹¹]-SP, an antagonist to substance P (SP), but not by ganglion blockers, leading these workers to infer that these responses were due to the release of SP via antidromic stimulation of vagal afferents. Guinea-pig lungs receive sensory innervation containing SP or SP-like immunoreactive material (Nilsson et al., 1977; Wharton et al., 1979; Lundberg et al., 1984), and capsaicin given systemically greatly reduces the SP-like immunoreactivity of pulmonary afferents (Lundberg & Saria, 1983; Lundberg et al., 1983a, 1984). Recently, it has been shown that eledoisin, physalaemin, kassinin, and substance K are present in guinea-pig airways (Lazarus et al., 1982; Watson, 1984; Hua et al., 1984, 1985). These tachykinins are a group of naturally occurring peptides that possess a similar C-terminal pentapeptide sequence, Phe-X-Gly-Leu-Met-NH₂, where X is a hydrophobic or aromatic residue (Fig. 8). They are known collectively as tachykinins because of their ability to produce a rapid contraction of smooth muscle (Erspamer, 1981). In the last few years, several reviews have appeared on tachykinin receptors (Brown & Hill, 1983; Harmar, 1984; Regoli et al., 1984; Watson, 1984; Jessell & Womack, 1985; Quirion, 1985). Erspamer (1981) first suggested that there is more than one type of tachykinin receptor in vertebrates. This notion was extended by Iverson et al., (1982) and Buck et al., (1984). In the absence of potent, selective antagonists to SP (Karlsson et al., 1984; Post et al., 1985), tachykinin receptors have been differentiated by assessing the relative potencies of various tachykinins on different tissues. On this basis, Iverson et al., (1982) suggested that there are two major types of tachykinin receptors. Buck et al., (1984) identified a third type of tachykinin receptor. Thus, at present, three types of tachykinin receptors are known — SP-P, SP-E, and SP-K (Table 4) (Buck & Burcher, 1986). This concept of multiple tachykinin receptors is supported by results obtained by using SP-fragments and SP-analogs (Table 4) (Regoli et al., 1984).

Substance P	Arg - Pro - Lys - Pro - Gln - Phe - Gly - Leu - Met-NH ₂
Physalaemin	pG1 - Ala - Asp - Pro - Asn - Lys - Phe - Tyr - Gly - Leu - Met-NH ₂
Eledoisin	pG1 - Pro - Ser - Lys - Asp - Ala - Phe - Ile - Gly - Leu - Met-NH ₂
Eledoisin-related peptide	Lys - Phe - Ile - Gly - Leu - Met-NH ₂

Figure 8. Amino-acid sequence of selected tachykinins.

Table 4: Putative Classes of Tachykinin Receptors

Characteristic	SP-P type	SP-E type	SP-K type	References
Potency of homologues	PH>SP>SK>EI>Kass>NK	SK>Kass=EI>NK>SP>PH	NK>Kass>EL>PH>SK>PH	Lee et al., 1982; Brown & Hill, 1983; Buck et al., 1984; Watson, 1984; Quirion, 1985.
Potency of fragments	SP(3-11)>SP(4-11)>SP(6-11)	Not determined	SP(4-11)>SP(3-11)>SP(6-11)	Regoli et al., 1984; Quirion, 1985.
Antagonists	Non-selective	Not determined	Non-selective	Regoli et al., 1984
Localization in peripheral tissues	Guinea-pig ileum, urinary bladder, and vascular smooth muscle	Rat vas deferens, hamster urinary bladder, guinea-pig trachea	Not determined	Lee et al., 1982; Watson, 1984.

Abbreviations: EI: eledoisin; Kass: kassinin; NK: neuromedin K; PH: physalaemin; SK: substance K; SP: substance P.

Recently, it has been shown that SP has an inhibitory effect on nicotinic responses (Boyd et al., 1983). This effect is dependent on the conservation of both N- and C-terminal amino acid sequence. Moreover, this action of SP is not mimicked by physalaemin or eledoisin, suggesting that the effects are not mediated by interaction with SP-P, or SP-E, or SP-K receptor sites. Rougon et al., (1983) using cultured astrocytes, showed that SP enhanced the ability of β -adrenergic agonists to increase levels of cAMP. At present it is unclear whether this enhancement of β -adrenergic responses is mediated via activation of tachykinin receptors or by some other mechanism (see Jessel & Womack, 1985).

1.7 Mediators in Asthma

The chemical mediators are released primarily from mast cells (Holgate et al., 1986), but other cells, e.g., basophils, alveolar macrophages, are also involved in mediator release to varying degrees. The mediators can be divided into two groups — preformed (granule-associated) and newly generated (membrane-derived) (Table 5).

1.7.1 Pre-formed Mediators

The pre-formed, or granule-associated, mediators include histamine, the peptides that comprise ECF-A, HMW-NCF, and various neutral proteases, acid hydrolases, and heparin proteoglycan.

In man, histamine is contained mainly in mast cells and basophils and it is derived from histidine by the enzymatic action of histidine decarboxylase. Histamine has both direct and indirect effects on human and guinea-pig airways (Biggs, 1984). Direct effects of histamine may be either contractile or relaxant (Chand & Eyre, 1975). The contractile effects of histamine involve H_1 receptors and are blocked by 'classical' H_1 -antagonists (Ash & Schild, 1966; Chand & Eyre, 1975). The relaxant effects of histamine involve H_2 receptors and are blocked by H_2 -antagonists such as cimetidine (Brimblecombe et al., 1975; Chand & Eyre, 1975). The distribution of H_1 - and H_2 -receptors within the lungs varies among species (Eyre,

Table 5: Mast cell derived mediators.

<u>Preformed Mediators</u>	
Amines	Histamine
Neutral proteases	Tryptase Carboxypeptidase B Kininogenase Dipeptidase Chymotrypsin
Exoglycosidases	β -hexosaminidase B β -glucuronidase β -galactosaminidase Arylsulfatases B
Chemotactic factors	Eosinophil chemotactic factor of anaphylaxis (ECF-A) High molecular weight neutrophil chemotactic factor (HMW-NCF)
Proteoglycan	Heparin (mw 60,000)
<u>Secondary or Newly Generated Mediators</u>	
Prostaglandins	PGD ₂ , PGF ₂ α , PGE ₂ , PGI ₂
Slow reacting substance of anaphylaxis (Leukotrienes)	LTC ₄ , LTD ₄ , LTE ₄
Thromboxanes	TXA ₂ , TXB ₂
Chemotactic factors	5-HETE, LTB ₄
Platelet activating factors	PAF-Acether (AGEPC)
Others	Prostaglandin-generating factor of anaphylaxis (PGF-A)

1973/ Chand & Eyre, 1977). The indirect actions of histamine are mediated through afferents and efferents in the vagus nerve (Nadel, 1980a, 1980b; Biggs, 1984). Histamine stimulates at least two kinds of mechanoreceptors in the lungs — 'irritant' receptors (Widdicombe, 1981) and 'J' receptors (Paintal, 1969). Afferents from both receptors cause reflex bronchospasm via vagal efferent pathways. In addition to bronchoconstriction, histamine induces mucus secretion via H_2 receptors from submucosal glands, and contributes to mucosal edema by increasing capillary permeability via H_1 receptors (Kaliner, 1985).

HMW-NCF attracts neutrophils to the site of inflammation, and ECF-A acts similarly on eosinophils. The time course of HMW-NCF release, from mast cells is similar to that of histamine. HMW-NCF is considered to be a better indicator of mast cell activation than histamine (Howarth et al., 1985). The appearance of HMW-NCF in circulation following mast cell activation can be blocked by pretreatment with membrane stabilizing agents like sodium cromoglycate (SCG) (Howarth et al., 1985).

1.7.2 Newly Generated Mediators

Newly generated, or membrane-derived, mediators are mostly arachidonic acid metabolites. Arachidonic acid is derived from phosphatidylcholine by the enzymatic action of phospholipase- A_2 . Arachidonic acid is then metabolized by cyclooxygenase to form PGs and thromboxanes (TXs), or by 5, 15-lipoxygenase to form mono-hydroxy fatty acids and the LTs (Fig. 9).

PGs have direct contractile and relaxant effects on the airways. $PGF_2\alpha$, PGD_2 , and TXA_2 contract, and PGE_2 and PGI_2 relax human airways in vitro (Hutas et al., 1981) and in vivo (Walters et al., 1982; Hardy et al., 1985). PGA_2 , PGD_2 , and $PGF_2\alpha$ increase mucin secretion in human airways, but PGE_2 reduces the secretion (Nadel et al., 1985). PGD_2 , PGE_2 , and PGI_2 augment the increase in capillary permeability induced by histamine and bradykinin.

The main product of the lipoxygenase pathway is slow-reacting-substance of anaphylaxis (SRS-A), which is composed of LTC_4 , LTD_4 , and LTE_4 . These constituents of

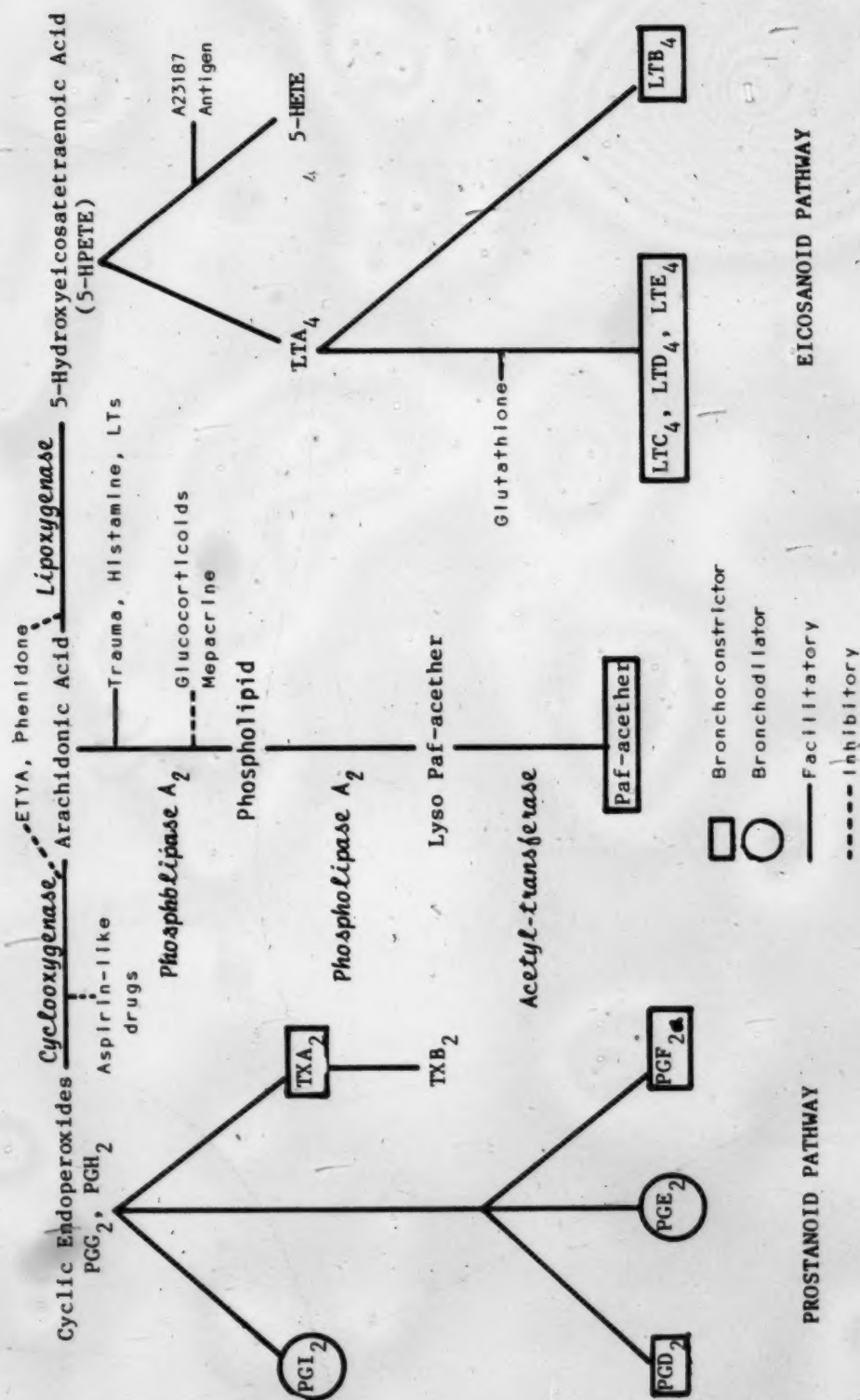


Figure 9. Pathways for newly generated mediators.

SRS-A exert profound effects on the tracheobronchial tree in vitro and in vivo (Hedqvist et al., 1980; Borgeat & Sirois, 1981; Goetzl, 1981; Lewis & Austen, 1981; O'Flaherty, 1982; Piper, 1983, 1985). In guinea pig and man, LTs are more potent in inducing bronchospasm (Kay, 1983) and have a longer duration of action than histamine or PGs (Drazen et al., 1980, 1982), and are most active on the peripheral airways (Leitch, 1985). In some smooth muscle systems, rank order of potency in vitro is $LTD_4 > LTC_4 > LTE_4$. LTD_4 and LTC_4 are also powerful secretagogues for mucus (Shelhamer et al., 1980). In man and guinea pigs, the more polar LTs, particularly LTB_4 , are potent chemotactic agents (Ford-Hutchinson et al., 1980).

The effects of eicosanoids can be eliminated by blocking their synthesis or by applying specific receptor antagonists. In guinea-pig airways, cyclooxygenase can be blocked irreversibly by ASA (Vane, 1971) and reversibly by indomethacin (Burka & Paterson, 1980), causing diversion of arachidonic acid metabolism into the lipoxygenase pathway (Adcock & Garland, 1980; Burka & Paterson, 1980). In various tissues and cells including guinea-pig lung and mast cells, 5-lipoxygenase activity is blocked by 1-phenyl-3-pyrazolidone (phenidone) (Blackwell & Flower, 1978), nordihydroguaiaretic acid (NDGA) (Morris et al., 1979), and U-60,257 (piriprost) (Bach et al., 1982). The effects of LTs can be blocked by the FPL-55712. FPL-55712 is more effective against LTD_4 than LTC_4 -induced contractions, suggesting that LTC_4 and LTD_4 probably act at different receptor sites (Drazen et al., 1980). Separate binding sites for LTC_4 and LTD_4 have been demonstrated in membranes of the guinea-pig lungs using radioligand binding studies (Pong & DeHaven, 1983; Bruns et al., 1983).

PAF is another membrane-derived mediator. Membrane-derived phospholipid is converted to lyso PAF-acether, a precursor of PAF, by the action of phospholipase-A₂ (Fig. 9) (Vergafig & Benveniste, 1983; Page et al., 1984). The proportions of arachidonic acid and lyso compounds released seem to vary among different classes of cells. Alveolar macrophages are the main source of PAF, a potent bronchoconstrictor that also causes leukocyte chemotaxis and aggregation, and increases capillary permeability (Vergafig & Benveniste, 1983; Page et al., 1984; Morley et al., 1985).

1.8 Asthma as a Disease

1.8.1 Definition and Types

Asthma is a disease characterized by increased responsiveness of the airways to various stimuli, and is expressed clinically as recurrent episodes of generalized airways' obstruction which resolves either spontaneously or with treatment (Am. Thor. Soc., 1962). Clinically patients with asthma can be divided into three groups: 1) allergic or extrinsic asthma, associated with an allergic diathesis; 2) cryptogenic, intrinsic, or non-allergic asthma, not associated with classical immediate hypersensitivity reactions; 3) chronic obstructive lung disease (COLD) with a reversible component to the airways' obstruction.

Extrinsic or allergic asthma usually appears in childhood. It is associated with high circulating levels of reaginic antibodies (IgE) directed against common environmental antigens, such as house dust mites (*Dermatophagoides pteronyssinus*), pollens, animal danders, and mould spores. Patients' skin tests are usually positive for those specific antigens (Holgate, 1983; Aas, 1985). The prevalence of allergic rhinitis, food allergies, conjunctivitis, and atopic eczema is increased in allergic asthmatics. However, not all allergic asthmatics are atopic (Rees, 1984).

Cryptogenic or intrinsic asthma usually occurs later in life — "late onset asthma" — though it occurs occasionally in children. It is not associated with the clinical or immunological markers of allergy and is more resistant to treatment than the allergic form of the disease (Holgate, 1983).

Some patients with COLD develop a reversible component to their airways obstruction and become "functionally" asthmatic. This form of asthma is often termed "asthmatic bronchitis" in adults and "wheezy bronchitis" in children (Holgate, 1983). The increased airway responsiveness in these patients is believed to be related to repeated bacterial and viral infections.

1.8.2 Phases of Asthma

The events that follow an asthmatic episode in a susceptible individual can be divided into three phases: 1) rapid, spasmogenic; 2) late, sustained; and 3) subacute/chronic inflammatory (Kay, 1983). These phases are easiest to identify and distinguish in allergic asthma and hence the following description pertains to this.

The rapid spasmogenic phase is predominantly bronchospastic, develops rapidly, is maximal at 10-20 min, and resolves within 2 h. The time course of the rise in plasma histamine and HMW-NCF parallels the changes in airway resistance (Kay et al., 1985). This is a type I (anaphylactic) allergic reaction. The rapid phase is mediated largely through histamine — acting directly on smooth muscle or indirectly via vagal reflexes. SCG and H_1 -receptor antagonists administered before challenge inhibit the reaction, which is unaltered by giving corticosteroids (Kay, 1983). Isoproterenol administered after challenge completely reverses the immediate reaction.

After challenge, many subjects have a second, often more severe, rise in airway resistance that is maximal 6-8 h following exposure. Late-phase reactions are more sustained and have slower onsets and resolutions than rapid-phase reactions. The late phase has both inflammatory and bronchospastic components, and is invariably followed by enhanced 'non-specific' bronchial hyperreactivity, that may persist for days (Cartier et al., 1982; Cockcroft, 1984). Late reactions are inhibited by prior administration of SCG or corticosteroids (Cockcroft, 1983), but are not well reversed by isoproterenol acutely administered.

Over a period of time untreated, or partially treated, asthma enters a subacute or chronic inflammatory phase, that is characterized by infiltration of the bronchi with eosinophils and mononuclear cells, presumably recruited by ECF-A and LTB_4 . This chronic phase is usually, but not always, responsive to corticosteroids.

1.8.3 Pathophysiology of Asthma

Despite differences in clinical patterns and associated factors, the pathological features of the various types of asthma are remarkably similar. Microscopically, there is destruction of the bronchial epithelium, mucosal edema, hypersecretion of mucus, infiltration of the airway wall with eosinophils and neutrophils, thickening of the subepithelial basement membrane and hypertrophy of airway smooth muscle (Hogg et al., 1977).

Many of the pathological features can be accounted for by the release of inflammatory mediators, that in the case of allergic asthma are mast cell derived. These mediators do not appear to be the cause of asthma because they have little effect in healthy subjects (Nadel, 1980b). Allergy cannot be the sole cause because many patients with evidence of allergic disease, e.g., patients with nasal allergies, have no clinical pulmonary manifestations, despite the fact that inhalation of specific allergens causes airway narrowing and, many (especially older) patients with asthma have no evidence of allergy. Nevertheless, all patients with asthma have a sign in common — bronchial hyperreactivity (Boushey et al., 1980). Hyperreactivity is a state of enhanced bronchoconstrictor responsiveness of the airways to a variety of heterogeneous stimuli, e.g., 1) pharmacologic and immunologic aerosols (methacholine, histamine, $\text{PGF}_2\alpha$, LTs, and antigens); 2) physical or chemical agents (ozone (O_3), sulphur dioxide (SO_2), nitrogen dioxide (NO_2), distilled water, and hypertonic solutions); 3) thermal factors (exercise, hyperventilation, cold air); 4) infections (common cold, influenza, other viruses); and 5) emotions. Although hyperreactivity appears fundamental to the pathogenesis of asthma, it may also occur in the absence of clinical asthma, e.g., after viral infections of the respiratory tract, and after exposure to O_3 or toluene di-isocyanate (TDI). The physiological and clinical overlaps contributing to bronchial asthma are best set out in a Venn diagram (Fig. 10).

Studies (Douglas et al., 1973; Snapper et al., 1978; Weiss et al., 1981) have shown that asthmatics may comprise a hyperreactive extreme of a unimodal distribution, rather than a separate and distinct population. This concept allows one to visualize the possibility of normal individuals developing changes in the reactivity of their airways that could move them

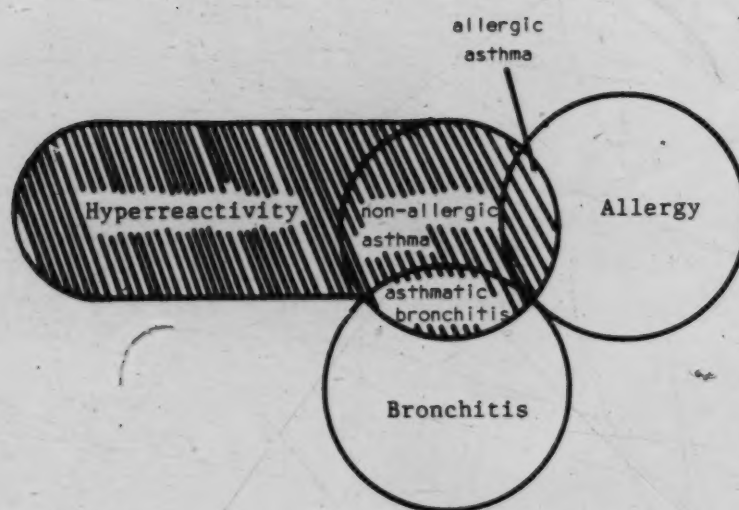


Figure 10. Venn diagram representing the clinical, physiological, and immunological overlaps in asthma.

acutely or chronically into the asthmatic range. Furthermore, it implies that reducing the responsiveness of the tracheobronchial tree should benefit all patients. The cause(s) of hyperreactivity are unknown, but the degree of hyperreactivity, measured by histamine inhalation, correlates positively with the severity of the disease (Cockcroft et al., 1977).

Nadel and his colleagues observed that mechanical (Nadel & Widdicombe, 1962), chemical (Nadel et al., 1965), and pharmacological (DeKock et al., 1966) stimulation of the nerve endings in airway epithelium causes reflex bronchoconstriction that was abolished by cutting the vagi in animals (Nadel & Widdicombe, 1962; Nadel et al., 1965; DeKock et al., 1966), or by atropine sulfate in animals and in healthy human subjects (Nadel et al., 1965). These workers suggested that the exaggerated responses in hyperreactive patients might be due to exacerbation of these reflex responses (see Fig. 11) and that damage to the airway epithelium might be the mechanism by which these sensory receptors are "sensitized" (Nadel, 1973; Barnes, 1986). This concept of hyperreactivity leading to bronchospasm is illustrated in Fig. 11. Reflex bronchospasm could be initiated by a number of pathways. In infections (bacterial and viral), the receptors are sensitized by lysosomal enzymes either directly or via the inflammatory process. Lysosomal enzymes are released from both eosinophils and neutrophils. In 'intrinsic' asthma, the damage could be due to the infectious processes discussed above. In 'extrinsic' asthma, inflammation associated with an immunological response involving the release of mast cell mediators, might be involved. Alternatively, these receptors could be sensitized directly by damage to the airway epithelium (Nadel, 1980a).

Afferent receptors with both myelinated (Widdicombe, 1981) and unmyelinated (Coleridge & Coleridge, 1984) fibers are involved in reflex bronchospasm, and have been described in the mucosa of both large and small airways. The conclusion that stimulation of either type of receptor provokes bronchoconstriction is based on the observation that the agents that stimulate activity in the receptors also stimulate bronchoconstriction that is dependent on afferent and efferent neural pathways. This concept of "hyperreflexia" is discussed next.

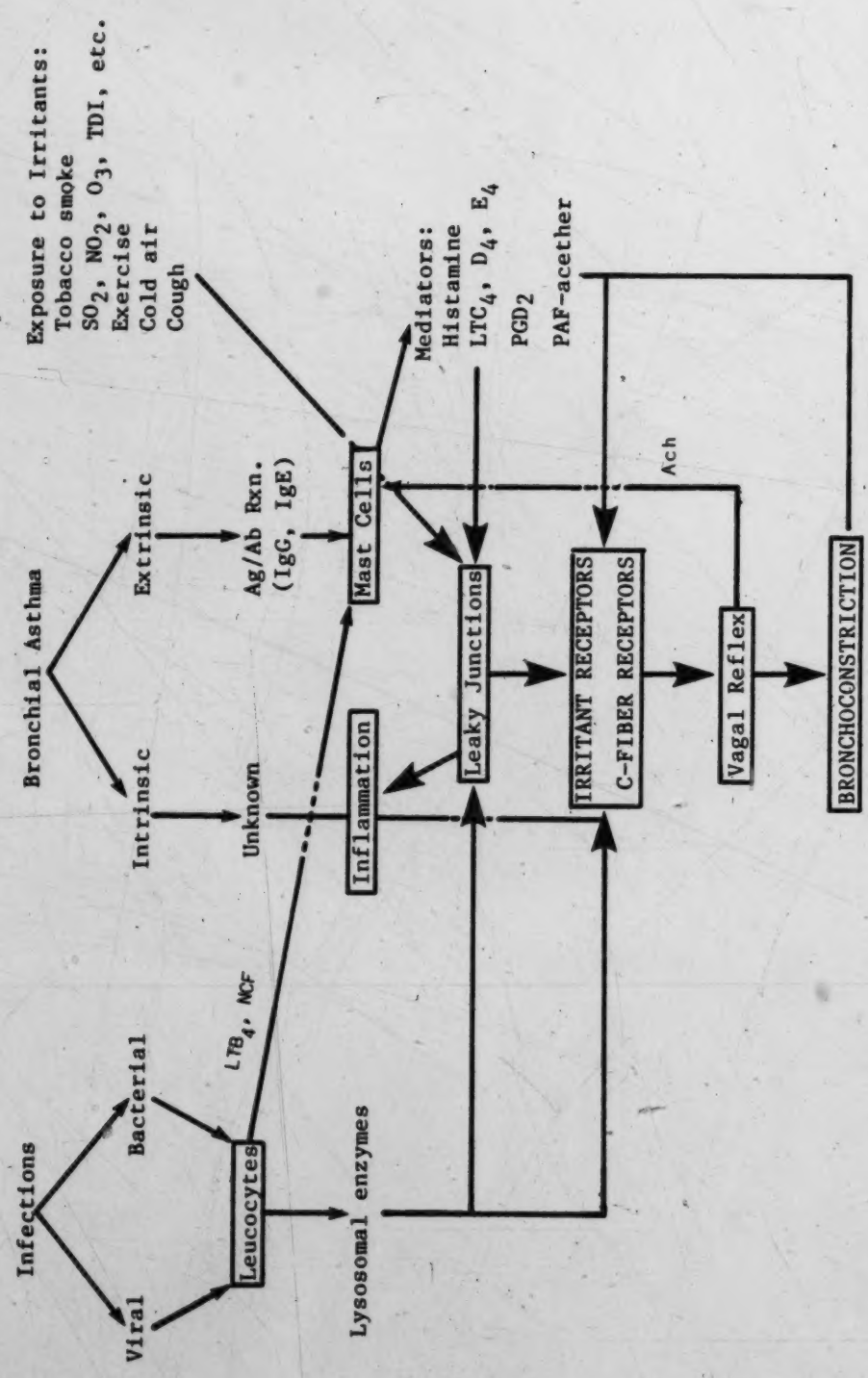


Figure 11. Possible pathways and interactions of pathophysiological mechanisms causing bronchoconstriction.

1.8.4 Hyperreflexia

Hyperreflexia as a cause of airway hyperreactivity has been discussed in a number of recent reviews (Boushey et al., 1980; Nadel, 1980a, 1980b; Widdicombe, 1981, 1985; McFadden, 1983; Nadel & Barnes, 1984; Boushey, 1985). Hyperreflexia is mediated via vagal afferents and efferents (Nadel, 1980a, 1980b; Widdicombe, 1981; Nadel & Barnes, 1984). These vagal reflexes can be set in motion not only by natural stimuli (see Section 1.8.3) but also by stimulating sensory receptors by the injection of chemical substances into the blood stream. The resulting effects are termed 'pulmonary chemoreflexes' and comprise a decrease in heart rate and blood pressure, apnea followed by rapid shallow breathing and bronchoconstriction (Barer & Nusser, 1953; Karczewski & Widdicombe, 1969b; Paintal, 1969).

Each receptor complex consists of neural and non-neural elements. The neural element consists of the unmyelinated terminal portion of the sensory fibers (both myelinated and unmyelinated) — usually termed the generator region, plus the adjacent central part, and the regenerative region where the propagated impulse is initiated (Paintal, 1964). In the case of myelinated fibers, the regenerative region is the first node of Ranvier, however, in unmyelinated fibers, this region is poorly defined. Evidence suggests that the diffusion barrier to injected chemical substances is weaker at the regenerative region of the ending than the diffusion barrier provided by the perineural sheaths in the main nerve (see Paintal, 1964). Paintal, (1971) suggested that all injected substances produce their effects by an action on the regenerative region. No substance is known that affects only a single type of sensory receptor and no receptor is known that is affected by only one chemical substance (Paintal, 1981). The endings of unmyelinated fibers are more sensitive to drugs than those of myelinated fibers. This difference in sensitivity is attributable to the regenerative region, which in case of myelinated fibers consists of narrow region of nodal membrane with limited area, while there is no such visible limitation in case of endings with unmyelinated fibers (Paintal, 1964).

The effects mediated via myelinated and unmyelinated fibers can be distinguished by inducing differential nerve block. Three methods — cooling, anodal polarization and compression — are used to block conduction selectively in the myelinated fibers of a nerve

trunk. The application of local anesthetics can be used to block conduction in unmyelinated fibers (see Coleridge & Coleridge, 1984). The selective block achieved by cooling and anodal polarization appears to depend on blockade of saltatory conduction (Paintal, 1965; Whitwam & Kidd, 1975). Nerve compression is rarely used to induce differential blocking except in studies of limb sensation in man (Torebjork & Hallin, 1974).

Cooling the vagus to 5°C will completely block activity in myelinated fibers leaving unmyelinated fibers unaffected. The unmyelinated fibers are blocked if the temperature is lowered to 0°C (Paintal, 1965). Recently, it has been shown that the selective blockade of A fibers at 6-8°C may be associated with a 60% reduction in the reflex activity of afferent C fibers (Roberts et al., 1981; Coleridge et al., 1982). This limitation of C-fiber discharge frequency when A fibers are blocked is less marked when anodal polarization is used for differential blockade. Therefore anodal polarization is thought to provide a more realistic estimate of the contribution of afferent vagal C-fibers to reflexes (Thorén et al., 1977).

The susceptibility of nerve fibers to local anesthesia is inversely related to the fiber diameter (Coleridge & Coleridge, 1984). The smaller fibers being blocked more readily as a consequence of their higher surface-to-volume ratio. The block is time dependent and is somewhat unreliable as a method for selectively blocking C fibers since small myelinated fibers, whose axonal diameters may be close to those of C fibers, are often blocked after shorter exposures than those required to block C fibers (Franz & Perry, 1974). Moreover, in the case of relatively thick nerve trunks, uneven penetration of the anesthetic makes differential block difficult to achieve (Franz & Perry, 1974).

Apart from differences in response to drugs arising from the differences between the regenerative regions of the endings of the myelinated and unmyelinated fibers, there are some useful differences with respect to their source of blood supply in lungs. Thus, injection of a drug, e.g., phenylbiguanide, into the aorta or left ventricle does not stimulate J receptors whereas injection of the same dose into the right side of the heart does (Paintal, 1969). Additionally, as there are no receptors in the lungs that receive blood only from the pulmonary circulation, it is possible to study reflexes involving J receptors by comparing the

effects of injecting the drug into the right and the left sides of the heart. However, the validity of conclusions drawn is reduced because of the anastomoses between pulmonary and bronchial circulations (discussed earlier in Section 1.5), and the existence of C-fiber afferents in the bronchial circulation (Coleridge & Coleridge, 1984). The reflex effects arising from bronchial C fibers can be distinguished from those arising from pulmonary C fibers (J receptors), by recording the time of onset of these reflex effects (Coleridge & Coleridge, 1984). Thus, the endings of bronchial C fibers are stimulated after a long delay by injection of chemicals into the right atrium, and after a shorter delay by injection into the left ventricle. In addition, bronchial C fibers are also stimulated by injection of small amounts of chemicals into the bronchial artery (Kaufman et al., 1980).

Pulmonary and bronchial C fibers differ not only in their vascular accessibility but also in the susceptibility of their afferents to a number of mechanical and chemical stimuli. In addition, there are distinct species-related differences in their sensitivity to various chemicals (Table 6). Despite the widespread use of the guinea pig in pulmonary research, studies on J receptors are lacking in this species. Many chemicals stimulate C-fiber afferents (Coleridge & Coleridge, 1984), but only two, phenylbiguanide and capsaicin, are used commonly in studies of afferent receptors and their associated reflexes (Karczewski & Widdicombe, 1969b; Paintal, 1969; Coleridge & Coleridge, 1984). Both are foreign chemicals with virtually no physiological or pharmacological significance apart from their action on sensory nerves. The use of two chemicals for studying the reflex effects of stimulating afferent endings relates to species differences in sensitivity of the unmyelinated afferents.

1.9 Mechanical Properties of the Lung

Only airway resistance and pulmonary elastance will be discussed (for review see Mead, 1961).

Table 6: Species Related Sensitivity of Lung C-fiber afferents to various chemicals.

Chemicals	Pulmonary C-fibers (type J receptors)						Bronchial C-fibers						References
	Cat	Dog	Rabbit	Rat	Man		Cat	Dog	Rabbit	Rat	Man		
Phenylbiguanide	+	0	+	+	0		+	+	+	+	0		2,3,7,8,9,14,16,18.
Capsaicin	+	+	0	+	0		ND	+	0	+	0		4,5,6,11,12,13,16,17.
Lobeline	+	+	ND	ND	+		0	ND	ND	ND	ND		9, 10.
Histamine	0	0	ND	ND	ND		+	+	ND	ND	ND		8, 10, 19.
Prostaglandins	ND	+	+	ND	ND		0	+	ND	ND	ND		19, 20.
Bradykinin	ND	0	ND	ND	ND		0	+	ND	ND	ND		10, 14, 16, 19.
Serotonin	+	0	ND	ND	0		0	ND	ND	ND	ND		1, 12, 14.

+ stimulated, 0 not stimulated, ND not determined

References: 1) Dawes & Comroe, 1954; 2) Paintal 1955; 3) Paintal, 1957; 4) Coleridge et al., 1964; 5) Coleridge et al., 1965; 6) Coleridge et al., 1968; 7) Karczewski & Widdicombe, 1969b; 8) Paintal, 1969; 9) Jain et al., 1972; 10) Paintal 1973; 11) Armstrong & Luck, 1974; 12) Coleridge & Coleridge, 1977; 13) Russel & Lai-Fook, 1979; 14) Kaufman et al., 1980; 15) Delepiere et al., 1981; 16) Sapru et al., 1981; 17) Coleridge et al., 1982; 18) Willette et al., 1982; 19) Coleridge & Coleridge, 1984; 20) Roberts et al., 1985.

1.9.1 Airway Resistance

Airway resistance (R) is defined as the ratio of the pressure difference (P) (between mouth and the alveoli) to the rate of flow ($\dot{V}$) i.e., $R = P/\dot{V}$. The pressure difference depends on the rate and the pattern of flow, and the latter could be — laminar, transitional, or turbulent. When the streamlines of flow are parallel to the sides of the tube, then it is called the laminar flow and it is usually present at low flow rates. At high flow rates, there is complete disorganization of the streamlines and therefore the flow is turbulent. Flow that is neither laminar nor turbulent is called transitional. The pressure flow characteristics of laminar flow are given by Poiseuille's law:

$$\dot{V} = \frac{P\pi r^4}{8nl}$$

where r is the radius, n viscosity, and l length. From the above relation it is clear that

$$P \propto \dot{V}$$

therefore, $P = K\dot{V}$ (K is a constant)

$$\text{since, } R = \frac{P}{\dot{V}}$$

$$\text{therefore, } R = \frac{8nl}{\pi r^4}$$

From this it is clear that reducing the radius by half will increase resistance 16-fold. Therefore, if baseline airway resistance is already high, then the bronchoconstrictor agents will have a greater effect. In turbulent flow, the pressure is approximately proportional to the square of flow rate:

$$\text{therefore, } P = K\dot{V}^2$$

In general the driving pressure is determined by both the flow rate and its square, i.e.,

$$P = K_1\dot{V} + K_2\dot{V}^2$$

The prevalence of a turbulent or laminar pattern of flow in any segment of the bronchial tree is determined by two variables: the air velocity (v), which depends on the rate of flow and cross-sectional area of the bronchial segment ($v = \dot{V}/\pi r^2$), and the kinematic viscosity of the gas mixture (ratio of specific gravity (d) to viscosity, (d/n)) (Hogg, 1982). The respective roles of these two factors are predicted by the Reynold's number (Re), which is given by

$$Re = \frac{2rvd}{n}$$

Flow is found to be laminar when the Re is less than 2,000, transitional between 2,000 and 3,000, and turbulent when the Re is above 3,000 (Hogg, 1982). From the expression above, it is clear that whenever the air velocity and the radius of the airway is large the Re is large, hence the flow is turbulent.

Gas flow in the trachea and large bronchi is rapid (high v and r , and therefore high Re) and therefore the flow is turbulent. As each airway branches, the sum of the cross-sectional areas of the daughter bronchi is greater than that of the parent bronchus. This increase in cross-sectional area reduces the velocity of air flow and also reduces the radius of individual bronchi - this reduces Re to make the flow transitional, but not enough to make it laminar. The fully developed laminar flow probably occurs in very small airways (West, 1979). The partitioning of airway resistance in healthy adults shows that the upper airway accounts for 50%, the central airways 45%, and the peripheral airways 5% of total pulmonary resistance (Strieder, 1976). Because large airways offer maximal resistance to air flow, any increase in airway resistance is most likely due to the constriction of the smooth muscle in the large airways.

1.9.2 Dynamic Thoracic Elastance

This term is used to describe the elastic properties of the lung and the chest wall. Elastance is the reciprocal of compliance which is defined as volume change per unit pressure change. As these parameters are usually measured without waiting for the airflow to become truly zero, they are best referred to as dynamic compliance and dynamic elastance. Dynamic elastance is dependent on the lung volume. At low lung volumes, the lungs are easy to expand therefore they have low elastance; at high lung volumes, the lungs are less easy to expand and therefore have high elastance. The elastance of normal chest wall is about the same as that of lungs. Elastance is increased in conditions like diffuse pulmonary fibrosis, pleural thickening, and atelectasis; whereas it decreases with old age and in persons suffering from emphysema. Constriction of the airway smooth muscle in peripheral airways will impair the outflow of the

air from the alveoli more than the inflow of the air into the alveoli. This is because of the phenomenon of "flow limitation". During expiration the pressure in an airway is smaller than in the alveoli, firstly because the flow is accelerated as it passes from the terminal units to airways of smaller total cross-sectional area; therefore the pressure falls by Bernoulli's theorem (see Pedley et al., 1977). Secondly, there is viscous energy dissipation as well, so that the pressure drop is even greater. Now at any lung volume the pressure outside an intrathoracic airway is effectively the same as pleural pressure, which is smaller than alveolar pressure by a fixed amount equal to the static recoil pressure of the lung at that lung volume. When the flow rate is great enough for the airway pressure to be smaller than pleural pressure, there will be a compressive stress on the airway, and it will tend to become smaller. If the compressive stress is great enough, the airway wall will buckle and collapse to a much smaller area. Collapse will, in turn, increase the resistance to flow. This is the basic mechanism for flow limitation.

Thus, bronchoconstriction in large airways primarily cause an increase in pulmonary-flow resistance (R). However, bronchoconstriction in small airways mainly affects the elastic properties of the lungs causing an increase in dynamic thoracic elastance (E) or decrease in dynamic thoracic compliance (C).

2. PROPOSED RESEARCH

2.1 Preamble

It is clear from the Introduction that 'bronchial hyperreactivity' is as fundamental to the pathogenesis of asthma as it is ubiquitous in asthmatics (see Fig. 10). Thus, knowledge of mechanisms behind airway hyperreactivity is essential for understanding the pathophysiology of asthma. Hyperreactivity does not appear to be due to inherent changes in airway smooth muscle. Smooth muscle from patients with airway hyperreactivity behaves similarly to muscle from the airways of normal patients, when examined in vitro (Zaid & Beall, 1966; Armour et al., 1984). Others have reported that differences exist between the physiological properties of 'normal' and 'hyperreactive' airway smooth muscle, however, these differences were not large (Simonsson et al., 1972). Bronchial hyperreactivity could result from a disorder of the regulation of airway calibre. Normally, airway calibre is influenced by reflexes originating from afferent sensory nerve endings that lie beneath tight junctions between epithelial cells in the airways. It is known that stimulation of two groups of pulmonary receptors — irritant and C-fiber receptors — induces reflex bronchospasm (see Fig. 11). Irritant receptors have myelinated afferents, and C-fiber receptors have unmyelinated afferents (see Table 1), afferents from each group are carried in the vagus. Despite the fact that the majority (about 80%) of vagal afferents arising from the lungs are unmyelinated (Kostreva, et al., 1975), myelinated afferents arising from irritant receptors have been most studied (see Widdicombe, 1981). We examined the roles of C-fiber afferents in causing reflex bronchospasm in guinea pigs.

2.2 Hypothesis

That airway hyperreactivity results from hyperreflexia mediated via airway afferent receptors, and that this hyperreactivity can be mimicked or induced by the administration of selective afferent receptor stimulants.

2.3 Objectives

Overall: To determine the role of unmyelinated vagal afferents in inducing reflex bronchospasm.

1. To establish a computerized technique for recording pulmonary-flow resistance (R) and dynamic thoracic elastance (E) or dynamic thoracic compliance (C).
2. To develop techniques for injecting drugs into ascending aorta or the aortic arch (i.a.), and the dorsal aorta in guinea pigs. Injection i.a. will result in high concentrations of drug in the vicinity of systemic chemo- and baro-receptors while bypassing J receptors, and injection into the dorsal aorta will bypass all thoracic receptors.
3. Using selective agonists — phenylbiguanide, sodium cyanide (NaCN), lobeline, nicotine, 1,1, dimethyl-4-phenylpiperazinium iodide (DMPP), 5-H.T., and capsaicin — to determine whether it is possible to stimulate C-fiber afferents in the lungs that form part of a reflex bronchospastic pathway, and to characterize the responses to these agonists by using selective antagonists, and nerve sections.
4. To characterize airway responses to vagal nerve stimulation by using selective antagonists.

2.4 Animal Model

The guinea pig was used as the animal model in these experiments because:

1. The smooth muscle in guinea-pig airways is abundant and extends into the alveolar ducts (Miller, 1947), making the airways especially sensitive to bronchoconstrictor agents.
2. Guinea pig's sensitivity to common agonists, peptides, and mediators of asthmatic bronchospasm is similar to that of man.
3. The pattern of the sympathetic, parasympathetic, and NANC innervation is similar in guinea pigs and in man (Richardson, 1979).
4. Most of the drugs used to treat asthma in man were developed in guinea pigs.

3. METHODS AND MATERIALS

3.1 Animal Preparation

Hartley strain guinea pigs (Charles River Canada Inc., St. Constant, Quebec) of either sex, weighing between 400-900 g, were anesthetized with urethane (1.25-1.75 g/kg) given intraperitoneally (i.p.). Some experiments were performed on animals anesthetized with pentobarbital sodium (40-50 mg/kg), or chloralose solution (80-100 mg/kg), injected i.p.

A cannula (PE 240) was inserted into the trachea and it was connected via a pneumotachograph (Fleisch 0000) to a Harvard small animal respiration pump for positive pressure ventilation (Fig. 11-1). Animals were ventilated at a tidal volume of 10 ml/kg, and at a pump speed of 20-25 strokes/min. The pneumotachograph was connected to a Validyne MP 45-14 or Grass PT5A volumetric transducer to measure flow rate ($\dot{V}$) (Fig. 11-1). A side arm of the tracheal cannula was used to monitor intratracheal pressure (P) via a Grass PT5A pressure transducer (Fig. 11-1). The flow of air into and out of the system was regulated by two separate valves.

3.2 Determination of pulmonary flow resistance and elastance

A MINC 23 computer was used to compute breath-by-breath values for R and E or its reciprocal, C, from flow rate and pressure signals using equations derived from Uhl & Lewis (1974).

3.2.1 Recording of pressure and flow rate signals

Inspiration occurs when the piston of the pump moves from bottom dead center (BDC) to top dead center (TDC) (Fig. 11-2). During inspiration both inlet and outlet valves are closed (Fig. 11-2) and the air from the pump enters the guinea pig's lungs via the pneumotachograph. During the whole inspiratory cycle, pressure across the pneumotach is recorded via an MP 45-14 transducer and pressure is measured by a PT5A transducer. Typical flow rate and pressure signals were recorded on a Grass 7D polygraph, and there were no

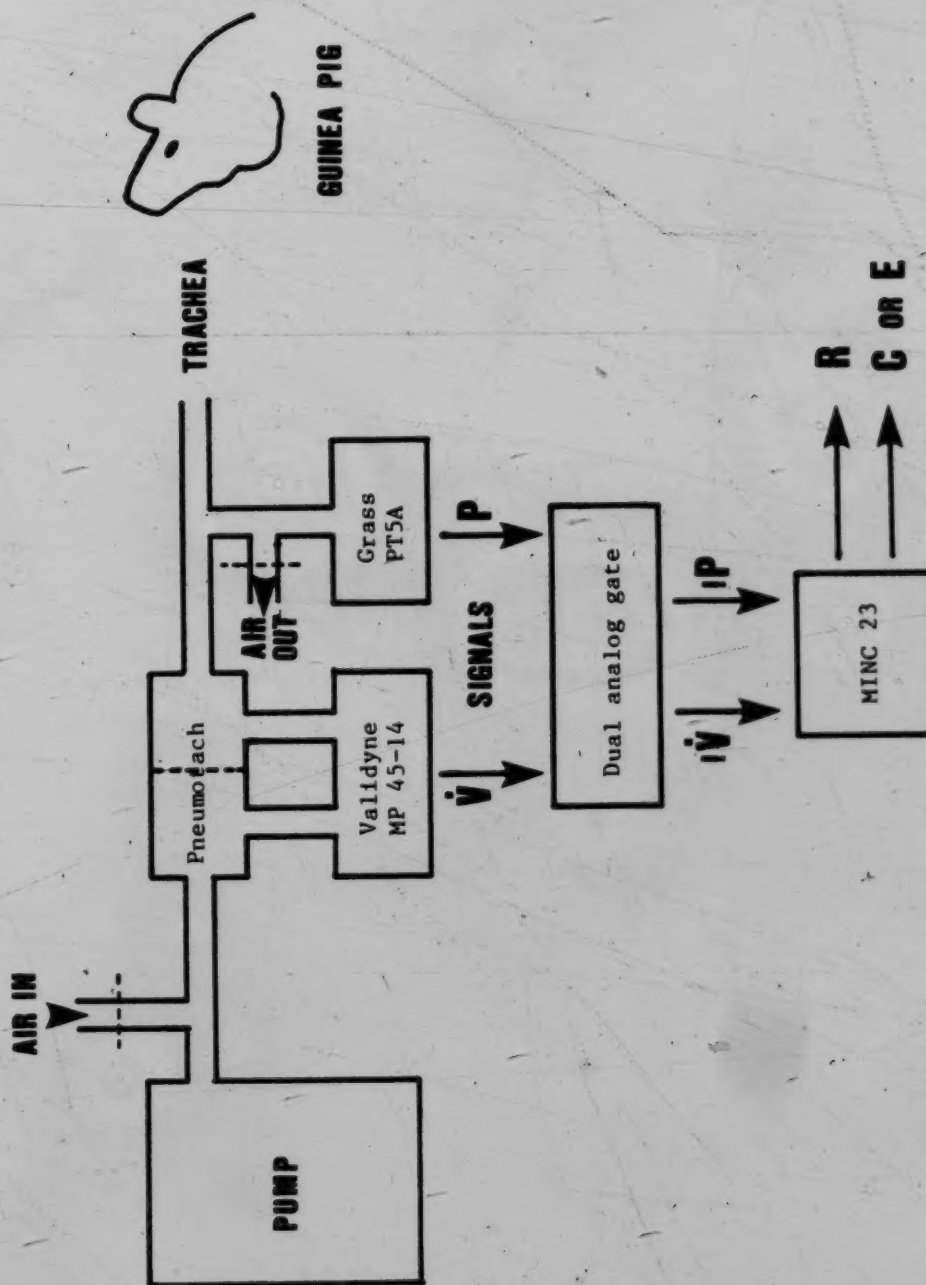


Figure 11-1. Experimental setup for measurements of R and E.

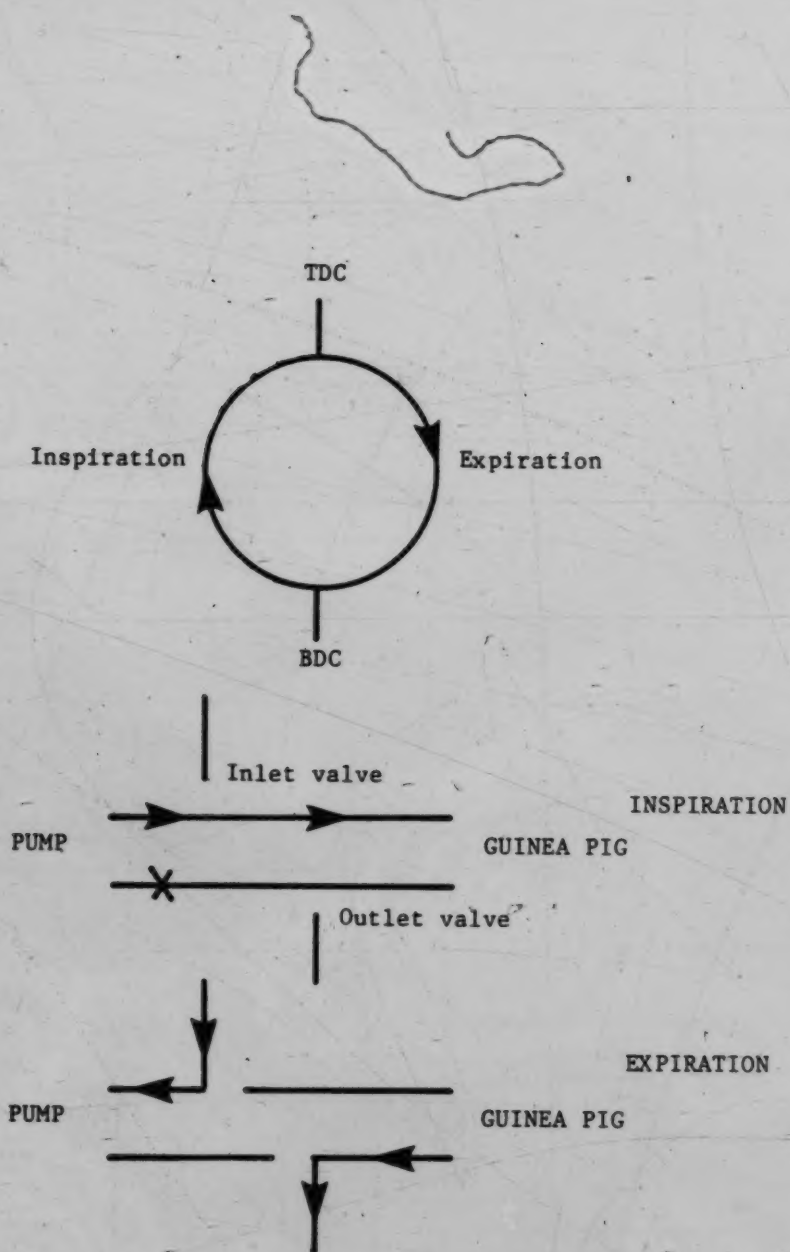


Figure 11-2. Relationship between piston movement and direction of air flow. TDC, top dead center; BDC, bottom dead center.

phase lags between the flow rate and pressure signals at the pump speeds used (Fig. 11-3). Although the ventilation is set at a constant volume, the volume of air actually injected is dependent upon the pressure. Boyle's law states that

$$P_1 V_1 = P_2 V_2, \text{ if } T \text{ is held constant}$$

where, P_1 & P_2 are the initial and final pressures; V_1 & V_2 are the initial and final volumes; and T is the temperature. Thus, any increase in pressure (ΔP) from P_1 to P_2 will lead to a corresponding decrease in volume (ΔV) of V_1 to V_2 . The relationship between volume and pressure in our experiments is shown in Fig. 11-4. Volume was derived from electronic integration of the flow rate signal, or by determining the area under the flow rate signal.

During expiration the piston moves from TDC to BDC and inlet and outlet valves open (Fig. 11-2). Opening of these valves is triggered electrically when the piston of the pump reaches the TDC. Room air is sucked into the pump via the inlet valve, and the air from guinea pig's lungs is exhaled passively via the outlet valve. During expiration there is no flow of air through the pneumotach and no pressure is recorded while the outlet valve is open, and no signals are recorded (or generated) either for flow rate or pressure. Expiration appears as 'baseline' on the polygraph recording (Fig. 11-3). In our experiments, inspiration and expiration were of nearly equal duration.

Tracheal pressure is used to estimate transthoracic pressure (P) during '*positive pressure ventilation*'. Calculations of elastance from these pressure measurements give an estimate of dynamic thoracic elastance (E) and not dynamic pulmonary elastance (E_L). Most of the animals were injected i.v. with pancuronium (0.1 mg/kg), in order to paralyze the respiratory muscles and prevent interference from spontaneous respiration and the thoracic muscles. At this dose level, pancuronium has no effect on measurements of R or C (Biggs & Peterson, 1980). The pump's solenoid-operated valves induce small signal artefacts, that were eliminated by passing the signals through a dual analog gate, making certain that the filtered signals included zero-flow points for both flow rate and tracheal pressure signals.

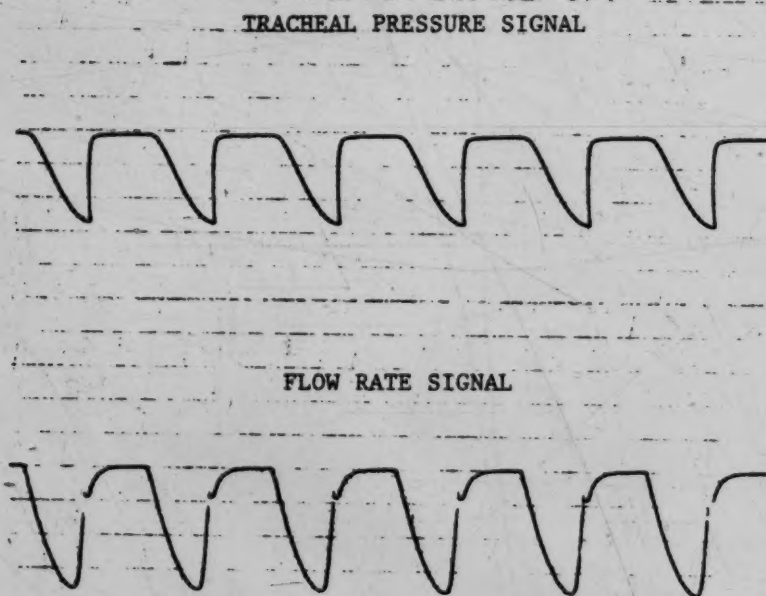


Figure 11-3. Typical traces of tracheal pressure and flow rate signals.

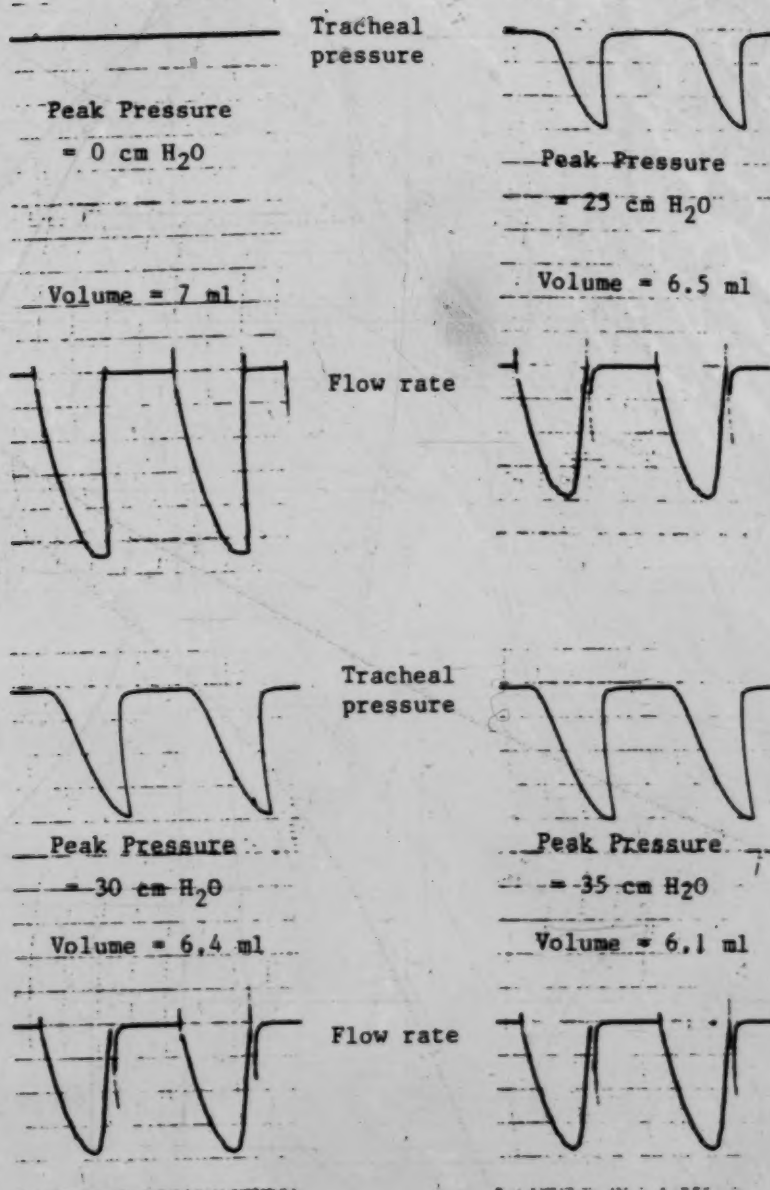


Figure 11-4. Traces showing the relationship between the tracheal pressure and the volume of air flowing through the pneumotach. The pump was set at a frequency of 20 cycles/min and at a volume of 7ml.

3.2.2 Data collection from flow rate and pressure signals

The computer program (written in BASIC) (see Appendix) converts the pressure and flow rate signals (shown in Fig. 11-3) into a digital array using analog to digital conversion. This array has 29,999 data points stored in time sequence in the computer's memory. 50 data points per second are collected for both flow rate and pressure signals up to a maximum of 5 min. After collection of data points, the computer goes back into its memory and separates the points into subgroups for every breath, i.e., inspiration. The method for breath identification is shown in Fig. 12. The computer scans forward in time in the digital array to find a positive data value point greater than a predetermined "level". Once such a point is identified, the computer assumes it is a potential breath and searches forward for a flow point equal to 0 flow rate to indicate the end of inspiration, it then returns to the original "level" point, and, by searching backwards it recognizes the zero-flow values at the start of inspiration. At a ventilation speed of 20 cycles/min, each inspiration lasts about 1.5 seconds (duration of inspiration and expiration being equal). So there will be about 75 data points (50 points/s) for each flow rate and pressure signal for each inspiration. For each inspiration, the computer calculates and consecutively stores the five summations: $\sum V^2$, $\sum PV$, $\sum V\dot{V}$, $\sum \dot{V}^2$, and $\sum P\dot{V}$. No smoothing was necessary in these experiments because of the large number of data points collected for calculations.

3.2.3 Calculations for R and E

The values for R and E were calculated for each inspiration by using the following equations.

Rohrer (1915) was the first to show that the volume-elastic and flow-resistive properties of the lungs could be analyzed by separating the pressure to which the lungs are subjected into its static and dynamic components. The relationship between pressure, volume and flow may be expressed algebraically. One such relationship used by Otis et al., (1956) is

$$\Delta P = K_{el} V + K_1 \dot{V} + K_2 \dot{V}^2 \quad (1)$$

where ΔP is the pressure difference across the lung, K_{el} is the ratio of pressure change to the

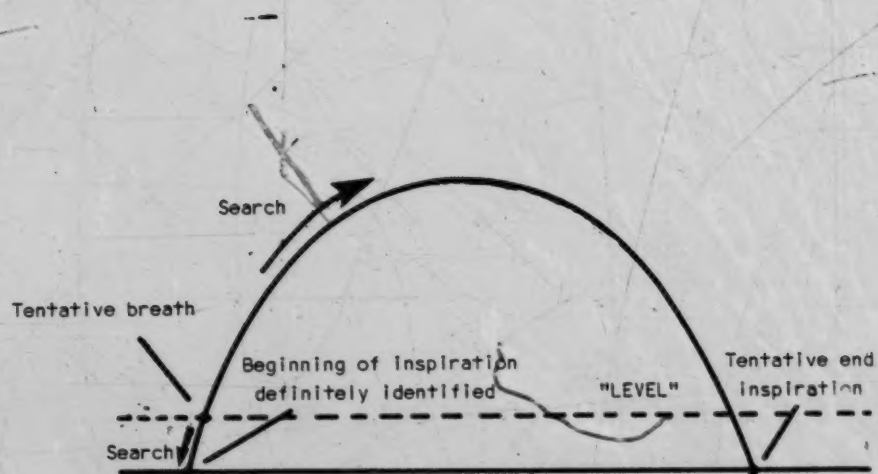
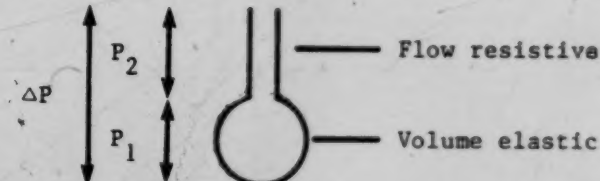


Figure 12. Schematic of computer logic for breath identification.

volume change (V) measured at the instant of no air flow, $\dot{V}$ is the gas flow (dV/dt), K_1 is the resistance, K_2 is the inertance, and $\ddot{V}$ is the gas acceleration (d^2V/dt^2). It is possible to construct a model with a single volume-elastic unit and a single flow-resistive unit (e.g. a balloon connected to a narrow glass tube) which conforms mechanically to expression (1), and indeed, for simplicity, it is sometimes useful to visualize the lungs as a whole in terms of such a system.

A single pulmonary pathway may be regarded mechanically as consisting of a volume-elastic part with a resistance, R , in series. By definition

$$C = \frac{V}{P_1}$$

$$R = \frac{P_2}{\dot{V}}$$


where V is the volume at a given pressure difference P_1 across the compliance, and $\dot{V}$ is the flow produced by a given pressure difference P_2 across the resistance. These equations are analogous to those defining electrical capacitance and resistance. If the total pressure across the system at any moment is ΔP then

$$\Delta P = P_1 + P_2$$

$$= \frac{1}{C} \cdot V + R \dot{V} \quad (2)$$

This expression is equation (1) expressed in terms of compliance and linear resistance ($K_1 = 1/C$; $K_2 = R$ and $K_3 = 0$). K_3 , or inertance is ignored because it accounts for <5% of the total pressure change even during heavy exercise (Mead, 1956). We therefore solve equation (2) for best-fit value of C and R during inspiration. Mathematically, least squares fitting minimizes the summed squared error between all observed points in the actual pressure wave and best-fit curve. We have a P best fit which minimizes the sum S of the squared error between the observed and best fit constants:

$$S = \sum [P(\text{best fit}) - P(\text{observed})]^2$$

since each observation

$$P(\text{observed}) = \frac{1}{C} \cdot V + R \dot{V},$$

$$S = \sum [P(\text{best fit}) - \frac{1}{C} \cdot V + R \dot{V}]^2$$

To minimize the error, we take partial derivatives $\Delta S / \Delta (1/C)$ and $\Delta S / \Delta R$, set them equal to zero, and solve for R and C (see Uhl & Lewis, 1974 for derivation).

$$R = \frac{\sum V^2 \sum P \dot{V} - \sum P V \sum V \dot{V}}{\sum V^2 \sum V^2 - (\sum V V)^2}$$

$$C = \frac{\sum V^2}{\sum P V - R \sum V \dot{V}}$$

$$E = \frac{\sum P V - R \sum V \dot{V}}{\sum V^2}$$

Using the above equations, values for R and E (or C) were calculated. This technique yields absolute values for R and E (or its reciprocal C) similar to those reported by others (Table 6-1).

3.2.4 Validity of the measurements

The validity of our system was tested using mechanical models in which the resistance (needles of different internal diameters) or the elastance (large sized balloon) were changed. The results (see Table 6-2) obtained with these alone or in combination show that the flow-resistive and the volume-elastic components of the lungs can be distinguished using this system.

Increases in R and E in our experiments occur due to changes in pressure and volume as discussed in Section 3.2.1 (see Fig. 11-4). Therefore, theoretically speaking a change in R or E could occur independent of the other if one is working with a pure flow-resistive or a pure volume-elastic system. But in practice, when measuring R and E in lungs, we do not have pure flow-resistive or volume-elastic components; therefore, the changes in R and E observed are dependent on each other to some extent. In most of the experiments reported in this thesis, increases in R and E occurred simultaneously, but there were some experiments in

Table 6-1. Comparison of absolute values for pulmonary-flow resistance (R), dynamic pulmonary compliance (C_L), and dynamic pulmonary elastance (E_L) obtained by various workers. All values were obtained in tracheotomized guinea pigs.

References	n	R [cm H ₂ O/(ml/s)] (mean $\pm$ S.D.)	C_L (ml/cm H ₂ O) (mean $\pm$ S.D.)	E_L (cm H ₂ O/ml) (mean $\pm$ S.D.)
Amdur & Mead (1958)	200	0.38 $\pm$ 0.16	0.24 $\pm$ 0.04	4.17*
Dennis et al., (1969)	200†	0.195 $\pm$ 0.02	0.214 $\pm$ 0.03	4.67*
MacLagan & Ney (1979)	23	0.044 $\pm$ 0.006	0.98 $\pm$ 0.18	1.02*
Ours	30	0.246 $\pm$ 0.06	0.357 $\pm$ 0.08 ‡	2.42 $\pm$ 0.6 ‡

* values for elastance were calculated from reported mean compliance values.

† The number of breaths in one animal, whose mean values for R and C are represented.

‡ We calculated values for dynamic thoracic compliance (C) and dynamic thoracic elastance (E) in contrast to calculation of C_L or E_L . Values for C and E were calculated in different set of animals.

Table 6-2. Comparison of absolute values for pulmonary-flow resistance (R), and dynamic thoracic elastance (E), obtained by using mechanical models. n is the number of breaths whose mean are the values shown under R and E.

Mechanical model	n	R [cm H ₂ O/(ml/s)] mean $\pm$ S.D.	E (ml/cm H ₂ O) mean $\pm$ S.D.
Balloon	10	0	7.416 $\pm$ 0.02
14 G needle	10	0.116 $\pm$ 0.002	0
14 G + balloon	10	0.155 $\pm$ 0.014	7.109 $\pm$ 0.036
16 G needle	10	0.168 $\pm$ 0.001	0
16 G + balloon	10	0.181 $\pm$ 0.001	7.237 $\pm$ 0.042
18 G needle	10	0.735 $\pm$ 0.003	0.179 $\pm$ 0.007
18 G + balloon	10	0.746 $\pm$ 0.003	7.410 $\pm$ 0.006
19 G needle	10	0.814 $\pm$ 0.004	0.307 $\pm$ 0.002
19 G + balloon	10	0.823 $\pm$ 0.006	7.724 $\pm$ 0.01

which increases in R and E occurred independently of each other. In vagal-stimulation experiments, increases in E lasted longer than increases in R, such that increases in E persisted even after R has returned to baseline (see Figs 38 & 39). Also, in some of the experiments with SP-antagonists reported in this thesis, increases in R were abolished without affecting increases in E (see Table 18 & Fig. 44). Both these findings support the conclusion that while changes in R and E occurred simultaneously in most experiments, they can be separated to some extent from each other.

Measurements of C (or E) and R provide a monitor of changes occurring in the small distensible, and large conducting airways, respectively (Nadel, 1965). A reversible decrease in C or an increase in E indicates constriction of the small airways and an increase in R suggests constriction of the large airways. We preferred to measure E rather than C because it is better to measure increases rather than decreases in a parameter. For practical purposes, the scale for measuring decreases is only 0 to 70-75 % though in theory it is 100%. Decreases in C lump together especially in the plateau region, i.e., from 50-70 % decreases, because decreases follow a reverse hyperbolic function. Thus, measuring E is better statistically (Clément & Woestijne, 1971).

The present method has a number of advantages over other non-computerized techniques: 1. We could examine all breaths for 5 min period, thus making it easy to judge the variability of E and R; 2. It eliminates observer bias in selection of breaths for calculations; and 3. this method is much less cumbersome and it provides a permanent record of E and R and does not require estimation of the angle of pressure-volume and pressure-flow plots on an oscilloscope.

Measurements were made as follows: the animals were ventilated at a stroke (lung) volume of 10 ml/kg for about 15 min to permit equilibration and adjustment of the system. Then the outlet valve was occluded and the animal's lungs were inflated once with two pump volumes. Normal ventilation was resumed; after equilibration for 2 min, followed by control measurements of R and E (or C), the drug was injected or the nerve stimulation performed during breath-by-breath R and E (or C) measurements. Two minutes after peak response, or

longer (up to 10 min) if the response persisted, the lungs were again inflated with two pump volumes and the system was allowed to equilibrate (>5 min) before the cycle was repeated. Baseline measurements of R and E (or C) made in this way showed $<5\%$ variation over 2 hr and consistently returned to "normal" values during experiments lasting up to 3 hours.

3.3 Measurement of Arterial Blood Pressure

In majority of the experiments, blood pressure (B.P.) was monitored from a carotid artery. However, in some experiments, B.P. was recorded from a femoral artery. The carotid or femoral arteries were cannulated using a PE 90 catheter with a tapered tip. Blood pressure was recorded on a Grass 7D polygraph via a Statham P23 Dd pressure transducer. The cannula was flushed intermittently with heparinized saline to prevent clotting.

3.4 Heart Rate

Heart rate was determined from the electrocardiogram (ECG) (QRS complex), using the equivalent of lead II in humans. The ECG was recorded on an HP polygraph, and heart rate was determined by counting the number of QRS peaks in a 10 s period.

3.5 Injection of Drugs

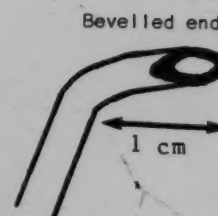
3.5.1 Injection into the superior Vena Cava or Right Atrium

A PE 50 catheter was passed down the right jugular vein a measured distance (~ 4 cm) until the tip was in the superior vena cava (SVC) or right atrium (RH). The catheter was secured in place; the position of its tip was confirmed at necropsy.

3.5.2 Catheterization of the Aorta

A PE 50 catheter with a bevelled end, was bent 1.0 cm from the tip (shown below) by dipping it in warm (150°C) mineral oil. This catheter was filled with heparinized saline (100 IU/ml) and connected to a P23 Dd pressure transducer which in turn was connected to a

Grass 7D polygraph to record carotid arterial pressure. The left carotid artery was preferred for cannulation for anatomical ease (see Fig. 13). Also, the shape of the catheter (bend at the end) helps in directing catheter's tip towards the heart and away from the descending aorta. The catheter was passed down the left carotid artery, such that the outer edge of the bend pointed away from the heart, until the tip entered the left ventricle (entry apparent in the pulse pressure monitored via the catheter) (see Fig. 14). Then, the catheter was withdrawn until the ventricular pulse disappeared and was replaced by an aortic pulse. The catheter was secured in place; its position was confirmed at necropsy. Drugs were injected via a disposable low-dead space 4-way stopcock (Intravalue®), while pressure in the aortic arch was recorded.



3.6 Surgical Procedures

3.6.1 Nerve Sections

The vagotomies were performed mid-cervically after the nerves were isolated and cleared of connective tissue. In the guinea pig, the cervical sympathetic nerve lies deeper than the vagus and thus this procedure did not represent vagosympathetic sectioning. The glossopharyngeal nerves were identified at the level of the jugular foramen, and were isolated and cleared. Just outside the jugular foramen the glossopharyngeal nerve divides, with the carotid-sinus-nerve branch going to the carotid-sinus and carotid-body regions. The glossopharyngeal nerve was sectioned central to its point of division so as to eliminate afferent input from carotid-sinus and carotid-body regions. In some experiments, the carotid-sinus nerve was sectioned to eliminate responses from carotid-sinus and carotid-body regions.

3.6.2 Decentralization

Animals were prepared by trephining the skull suboccipitally in the midline region, with a dental drill. Both common carotids were clamped. The dura was incised, and the brain

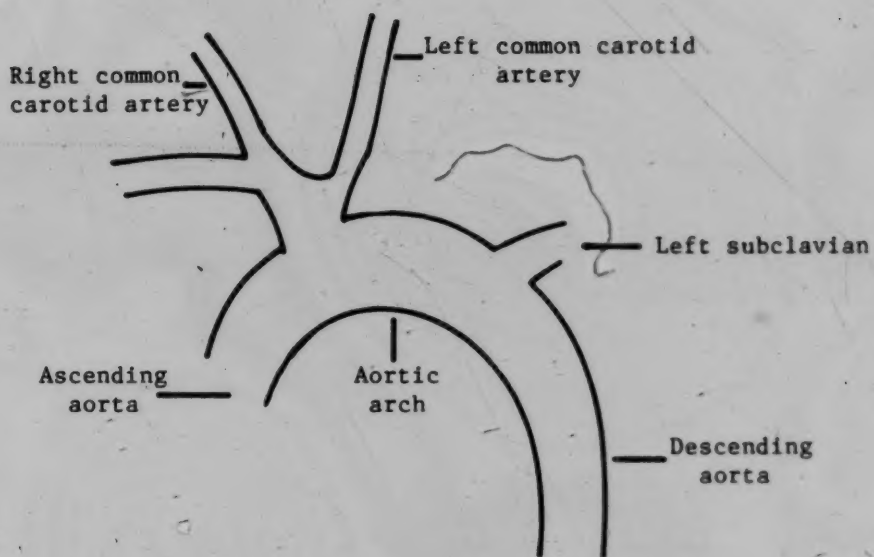


Figure 13. Anatomy of the aortic region in guinea pigs.

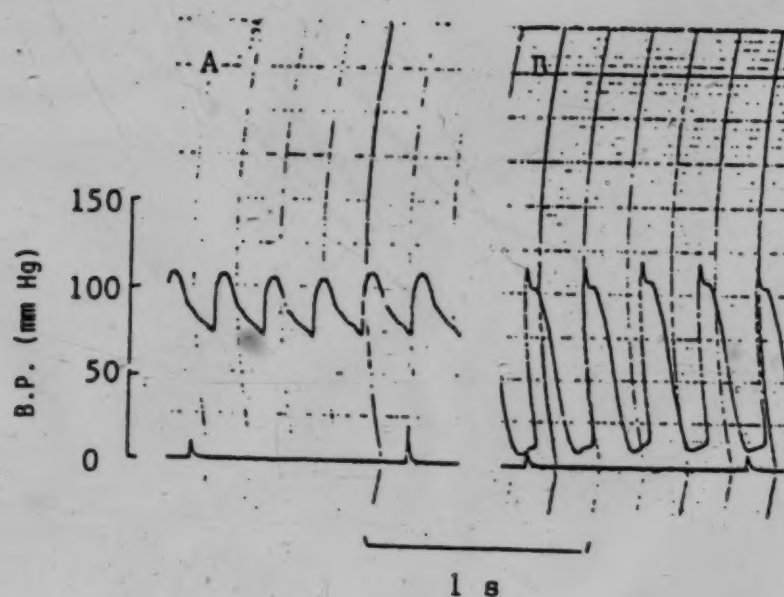


Figure 14.

Arterial blood pressure recordings from; A) aortic arch, and B) left ventricle.

sectioned just above C1 with a probe. Decentralization was considered to be complete when the animal showed the following signs: limb muscles became flaccid, spontaneous respiration stopped (due to destruction of respiratory centers in brain stem) and there was a drastic fall in arterial blood pressure to levels of 20–30 mm of Hg systolic (due to spinal shock). The hole in the skull was plugged by gently packing it with cotton wool and artificial respiration started. The head of the animal was kept elevated for a period of 10 min at which time the clamps on the carotid arteries were removed. The blood pressure stayed at 20–30 mm of Hg systolic during the 2–3 h period of the experiments.

3.6.3 Chemical sympathectomies

Sympathectomy was performed by injection of 6-hydroxydopamine (35–50 mg/kg) or reserpine (2.5 mg/kg) i.p., 24–48 h prior to experiments. Sympathectomy was considered complete when tyramine injected i.v., failed to induce any increases in B.P.

3.6.4 Stimulation of the Vagi

Both vagus nerves were isolated in the mid-cervical region after carefully separating them from the carotid artery (both lie within the carotid sheath). For central stimulation: one nerve was cut and the central end drawn into a bipolar platinum tunnel electrode; the other nerve was left intact. For unilateral peripheral stimulation: either a single vagus nerve was sectioned, and the peripheral end drawn into the tunnel electrode while keeping the other nerve intact, or both nerves were cut but only one stimulated (Fig. 15). For bilateral stimulation: both nerves were cut and their peripheral ends drawn into tunnel electrodes (Fig. 15). They were stimulated with pulse widths of 0.5 or 5 ms for 10–30 s at various frequencies (1.25–10 Hz), at supramaximal voltage (15 V), with a Grass S44 stimulator via a Grass SIU5 stimulation unit.

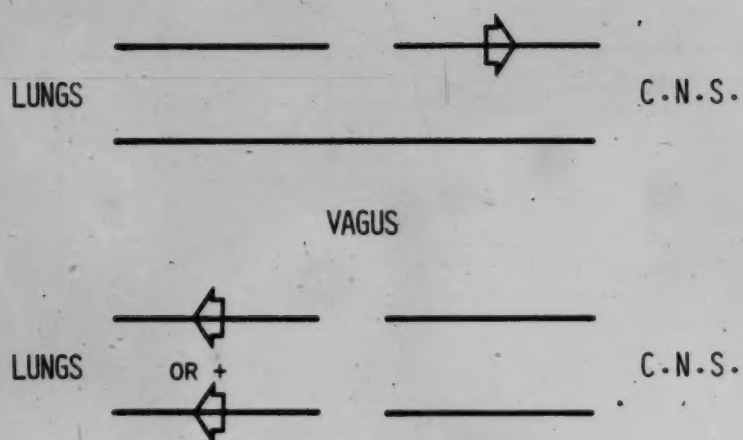


Figure 15.

Different ways of vagal stimulation. Arrow indicates direction of stimulation.

3.7 Drugs

All drugs were dissolved in or diluted with normal saline and injected i.v. and/or intra-aortically (i.a.). Whenever possible the drugs were injected in volumes less than 0.5 ml. Cannulae had a dead space of about 0.1 ml, and were flushed with 0.2 ml of saline after each injection.

3.7.1 Capsaicin Pretreatment

A stock solution was made up of 20 mg capsaicin, 0.2 ml 95% ethanol, and 0.1 ml Tween 80. This was diluted with 0.9% saline as required to give solution strengths of 5, 10, and 15 $\mu\text{g/ml}$, for i.v. or i.a. injection. When capsaicin was given as pretreatment (Lundberg & Saria, 1983), 50 mg/kg was injected subcutaneously (s.c.) 7 days before the experiments. Despite the concomitant administration of pentobarbital sodium (15 mg/kg, i.p.) or ketamine (40 mg/kg, i.p.) as anesthetic, in addition to aminophylline (10 mg/kg, i.p.) and salbutamol (0.05 mg/kg, s.c.), the mortality rate was 40–50%. All mortalities occurred within 8 h of injecting capsaicin and the animals surviving this period were apparently normal by next day. These surviving animals were studied 1 week later. In later experiments, mortality was reduced to <20% by giving the capsaicin in divided doses.

3.7.2 SP-Antagonists

SP-antagonists, dissolved in normal saline, were infused (i.v.) over 5 or 15 min, starting within 20 min after the injection of mepyramine (0.1 mg/kg) and atropine (0.1 mg/kg). Responses were recorded from 10 min until 60 min (after 5-min infusion) or from 30 min until 90 min (15-min infusions) after the injection of the SP antagonists. For [D-Arg¹, D-Pro², D-Trp^{7,8}, Leu¹¹]-SP, an additional dose of 0.3 mg/kg was injected immediately prior to injection of an agonist, or capsaicin, or electrical stimulation of the vagus nerves (see Lundberg et al., 1983b).

3.7.3 α -Chloralose

α -Chloralose was dissolved in distilled water at 37°C. α -Chloralose solution (10 mg/ml) was injected i.p. using a 18-gauge needle.

3.7.4 Other Drugs

All other drugs were dissolved or diluted with normal saline. The drugs used were: aminophylline injection (Glaxo Laboratories, Toronto, Ont.), atropine sulfate (B.D.H., Toronto), baclofen (gift from Geigy Pharmaceuticals, Mississauga, Ont.), bethanidine sulfate (gift from Burroughs Wellcome, Kirkland, P.Q.), capsaicin (Sigma Chemical Co., St. Louis, MO), α -chloralose (J.T. Baker Chemical Co., Phillipsburg, NJ), 1,1-Dimethyl-4-phenylpiperazinium iodide (Fluka AG, Buchs SG), eledoisin (Peninsula Laboratories, Belmont, CA), eledoisin-related peptide (Sigma), heparin sodium injection (B.D.H.), 6-hydroxydopamine (Sigma), ketamine hydrochloride (Parke, Davis Canada, Scarborough, Ont.), L-lobeline (Sigma), mepyramine maleate (Rhône-Poulenc Pharma, Montreal, P.Q.), mecamlamine hydrochloride (gift from Merck Sharp & Dohme, Kirkland), methysergide bimalene (Sandoz, Basle, Switzerland), morphine sulfate injection (Abbott Laboratories, Montreal), naloxone hydrochloride injection (Endo Canada, Montreal), nicotine dihydrochloride (J.T. Baker Chemical Co.), pancuronium bromide injection (Organon Canada, West Hill, Ont.), pentobarbital sodium (M.T.C. Pharmaceuticals, Hamilton, Ont.), pentolinium tartrate B.P. (May & Baker Ltd., England), 1-phenylbiguanide, 94% (Aldrich Chemical Co., Milwaukee, WI), physalaemin (Sigma), salbutamol sulfate (Allen & Hanburys, Toronto), serotonin creatinine sulfate (Sigma), sodium cromoglycate (gift from Fisons Corporation, Scarborough, Ont.), sodium cyanide (General Intermediates of Canada, Edmonton, Alta), [D-Pro²,D-Trp^{7,8}]-SP (Sigma or Peninsula), [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP (Peninsula), substance P (Sigma or Peninsula), trimetaphan camphorsulfonate injection (Roche, Montreal), tyramine hydrochloride (Sigma), and urethane (Sigma).

3.8 Analysis of data

At least four replicates were obtained in each group of experiments. Student's t-test was used to examine differences; differences were assumed significant at the 5% level. Results are expressed as difference from control, as a percentage of control.

4. RESULTS

4.1 Experiments with Phenylbiguanide

Phenylbiguanide was used to selectively stimulate C-fiber afferents (especially J receptors) in the lungs. Airway and cardiovascular responses to phenylbiguanide injected i.v. or i.a. were recorded and compared.

4.1.1 Effects on R and E

Phenylbiguanide (50–500 $\mu\text{g/kg}$), given i.v. or i.a. caused no significant changes in R and E in any of the animals studied ($n=36$).

4.1.2 Cardiovascular effects

The injection of phenylbiguanide (50–500 $\mu\text{g/kg}$) into the superior vena cava or right atrium caused a dose-dependent rise in mean arterial blood pressure (MABP), that lasted 2 to 5 min, depending upon the dose (Fig. 16). In general, the pressor response was proportional to the dose administered; increases in pressure in response to a single dose of $<200 \mu\text{g/kg}$ were dose-dependent and reproducible, but above this dose the response to repeated administration was enhanced even though MABP had returned to baseline in the interim. Similar doses of phenylbiguanide injected into the aorta caused an initial fall in MABP lasting 10–30 s, then a rise for 2–5 min (see Fig. 16). The effect was dose-dependent with single doses, and responses were enhanced as described above. Similar responses to phenylbiguanide were obtained in spontaneously respiring animals (Fig. 17). There were no significant alterations in heart rate ($P > 0.05$) in response to phenylbiguanide given by either route. In individual animals, pressor responses to phenylbiguanide injected i.a., were always smaller than with the same dose injected i.v.

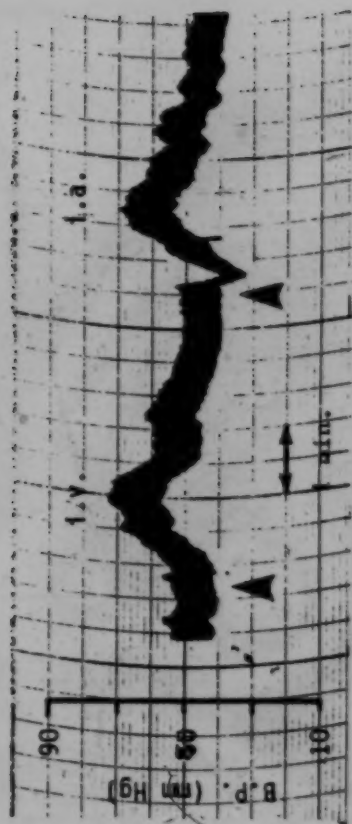


Figure 16. The effects of phenylbiguanide ($200 \mu\text{g/kg}$) injected i.v. or i.a. on B.P. in anesthetized paralyzed guinea pigs.

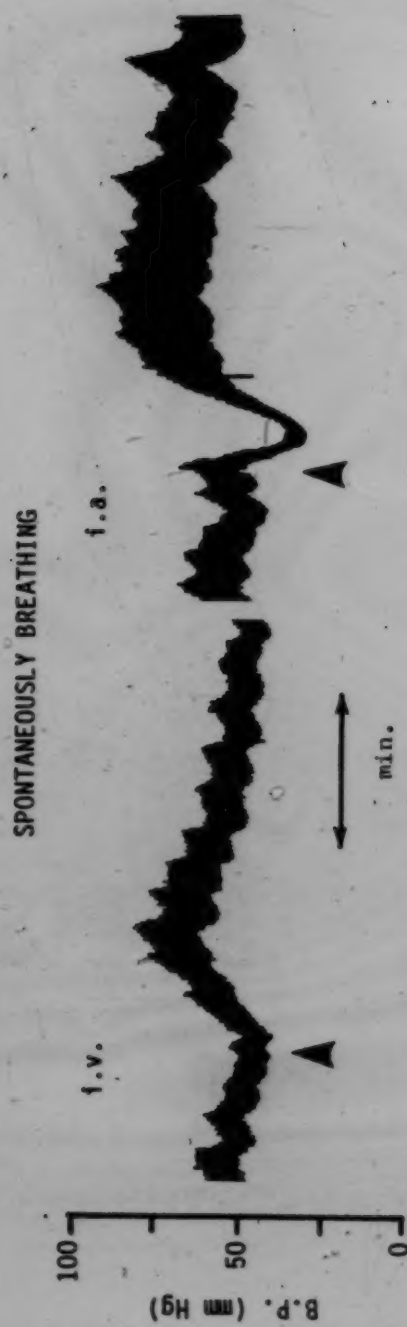


Figure 17. The effects of phenylbiguanide (200 μ g/kg) injected i.v. or i.a., on B.P. in spontaneously breathing guinea pigs.

4.1.3 Effects of nerve sections and decentralization

Bilateral vagotomy and sectioning carotid sinus nerves bilaterally significantly reduced the blood pressure response to the phenylbiguanide given by either route (Table 7). Combination of both nerve sections almost eliminated responses to phenylbiguanide given by either route. Phenylbiguanide, given i.v. or i.a., failed to induce any changes in blood pressure in decentralized animals (Table 7).

4.1.4 Effects of autonomic blockers

Atropine (0.1 mg/kg) significantly reduced the hypertensive effect of phenylbiguanide given i.v. or i.a., but had no effect on the depressor effect of phenylbiguanide injected i.a. Mepyramine (0.1 mg/kg), mecamlamine (2.5 mg/kg), and bethanidine (2.5 mg/kg) reduced both pressor and depressor responses to phenylbiguanide injected by either route (Table 8). Phenylbiguanide, given by either route, failed to induce any changes in MABP in 6-hydroxydopamine-treated animals. Sodium cromoglycate (10 or 20 mg/kg) greatly reduced pressor responses to phenylbiguanide given into either the right or left side of the heart and reduced (but not significantly) the depressor response to the drug given i.a. (Table 8).

4.2 Experiments with Sodium Cyanide

Reflex changes to NaCN, a selective chemoreceptor stimulant, were compared with those obtained with phenylbiguanide.

NaCN (50–500 μ g/kg), given into either the right or left side of the heart, induced no significant changes in R or E in any of the animals studied ($n=12$), but induced a rise in MABP. This lasted 2 to 5 min depending upon the dose (Fig. 18). In general, the pressor responses were proportional to the dose administered and increases in MABP to single doses of NaCN were reproducible. In individual animals, responses after i.a. and i.v. injections were of similar magnitude. The effects on heart rate were not recorded.

Rises in MABP induced by i.v. or i.a. administration of NaCN were significantly ($P < 0.05$) reduced by bilateral vagotomy and bilateral glossopharyngealotomy (Table 9).

Table 7: Effects of decentralization and nerve sections on the changes in mean arterial blood pressure in response to injection of phenylbiguanide into the superior vena cava (i.v.) or intra-aortically(i.a.).

Treatment	n	i.v.		i.a.	
		Pressor response (mm.Hg)		Depressor response (mm Hg)	
		Control	Test	Control	Test
Decentralization	4	N.A. [†]	0	N.A.	0
Bilateral vagotomy	6	8.8±1.5	1.8±1.2†	9.9±3.4	1.2±0.5†
Bilateral glossopharyngealotomy	6	8.4±2.1	2.5±1.0†	6.4±2.5	2.4±0.6
Both nerve sections	4	8.6±1.8	0.6±0.4†	7.2±2.7	0.6±0.2
				8.6±2.6	0†

† P = <0.05

N.A. - No control values obtained; see control values for the other treatments.

Table 8: Effects of various autonomic blockers on changes in mean arterial blood pressure in response to injection of phenylbiguanide into the superior vena cava (i.v.) or intra-aortically (i.a.).

Treatment	n	i.v.		i.a.	
		Pressor response (mm Hg)		Depressor response	
		Control	Test	Control	Test
Mecamylamine, 2.5 mg/kg	4	11.4±2.2	2.5±1.5†	9.0±4.0	3.5±1.7†
Bethanidine, 2.5 mg/kg	4	10.0±1.4	2.5±1.9†	4.8±2.8	0.7±0.6†
6-hydroxydopamine, 50 mg/kg	4	N.A.	0	N.A.	0
Atropine, 0.1 mg/kg	4	10.2±2.4	5.6±2.2†	8.6±2.3	8.9±2.5
Mepyramine, 0.1 mg/kg	4	11.5±1.6	4.3±2.1†	2.7±0.4	0.3±0.2†
Sodium cromoglycate, 10 mg/kg	6	9.5±1.4	1.9±1.2†	4.8±2.6	4.4±1.2
				9.3±1.0	3.7±1.7†
				8.5±0.6	5.4±0.3†
				7.3±0.2	0.1±0.1†

† P < 0.05.

N.A. no control values obtained; see control values for the other treatments.

Given 24 h before the experiment.

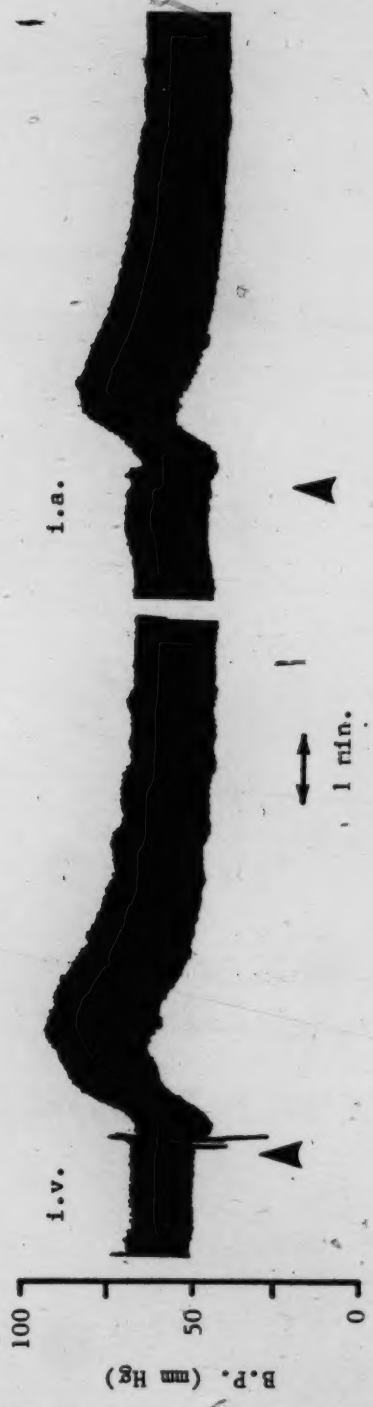


Figure 18. The effects of NaCN (500 µg/kg) injected i.v. or i.a., on B.P. in anesthetized paralyzed guinea pigs.

Table 9: Effect of various nerve sections on sodium cyanide-induced increases in mean arterial blood pressure (MABP). Results expressed as mean $\pm$ S.E.

Treatment	n	i.v.		i.a.	
		Control	Test	Control	Test
Bilateral vagotomy	4	10.2 $\pm$ 1.2	4.1 $\pm$ 1.0†	8.4 $\pm$ 1.1	3.1 $\pm$ 0.7†
Bilateral glossopharyngealotomy	4	11.4 $\pm$ 0.8	2.6 $\pm$ 0.5†	11.9 $\pm$ 2.3	3.2 $\pm$ 0.8†
Both nerve sections	4	12.7 $\pm$ 1.3	0‡	9.3 $\pm$ 1.7	0‡

† P = <0.05; ‡ P = <0.01.

Sectioning of both nerves completely abolished the pressor responses to NaCN.

4.3 Experiments with Lobeline

Lobeline was used to try and selectively stimulate C-fiber afferents (especially J receptors) in the lungs.

Lobeline (0.2–1.0 mg/kg) injected i.v. or i.a. induced insignificant changes in R, E, and MABP in all animals studied (n=5). Heart rate was not recorded.

4.4 Experiments with Nicotine

Nicotine was used to selectively stimulate C-fiber afferents in the lungs.

Nicotine (20–200 μ g/kg) injected into either the right or left side of the heart, caused no significant changes in R and E in any of the animals studied (n=4). Similar doses of nicotine injected i.v. or i.a., induced prompt rises in MABP, that lasted 30 s to 2 min (Fig. 19). Responses were dose-dependent and reproducible. In individual animals, the pressor responses to nicotine injected i.v. or i.a. were similar in size.

Increases in MABP induced by administration of nicotine i.v. and i.a. were not significantly altered by either bilateral vagotomy or mecamylamine (2.0 mg/kg) (Table 10). In animals pretreated with reserpine (4.0 mg/kg, i.p., 24 h beforehand), nicotine still induced increases in MABP (Table 10).

4.5 Experiments with DMPP

DMPP, a selective ganglionic stimulant, was used to compare changes in MABP obtained with nicotine.

DMPP (5–40 μ g/kg), injected into either the right or left side of the heart, induced no significant changes in R and E in any of the animals studied (n=6). The injection of DMPP i.v. or i.a., caused a rapid rise in MABP, that lasted 30 s to 2 min (Fig. 20). In general, the responses were dose-dependent and reproducible. In individual animals, pressor responses to DMPP injected i.a. were always smaller than when the same dose was

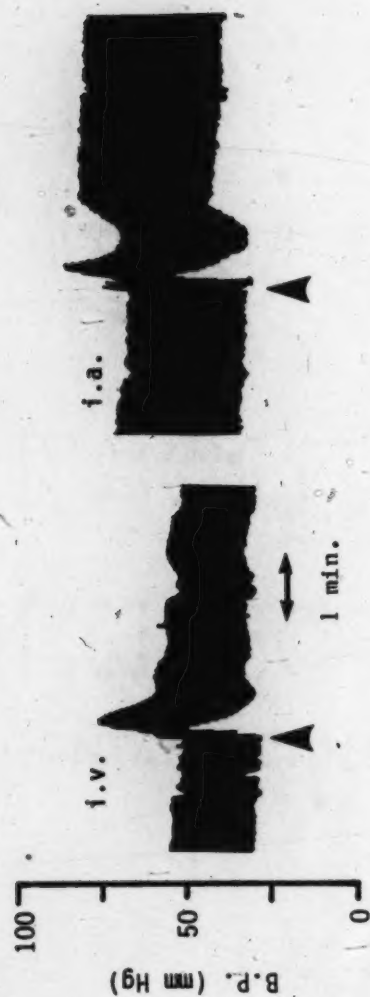


Figure 19. The effects of nicotine (200 $\mu\text{g/kg}$) injected i.v. or i.a., on B.P. in anesthetized paralyzed guinea pigs.

Table 10: Effect of various treatments on nicotine-induced increases in mean arterial blood pressure (MABP). Results expressed as mean $\pm$ S.E.

Treatment	n	i.v.		i.a.	
		Control	Test	Control	Test
Bilateral vagotomy	4	12.4 $\pm$ 1.2	11.7 $\pm$ 2.3	11.8 $\pm$ 1.7	13.1 $\pm$ 3.1
Mecamylamine, 2.0 mg/kg	4	10.4 $\pm$ 1.9	12.9 $\pm$ 2.0	12.6 $\pm$ 2.3	11.4 $\pm$ 1.8
Reserpine, 4.0 mg/kg	8†4	11.4 $\pm$ 2.1‡	11.8 $\pm$ 1.9	12.2 $\pm$ 2.5‡	9.7 $\pm$ 4.3

† Number of control experiments.

‡ Control values derived in previous experiments with the same dose of nicotine.

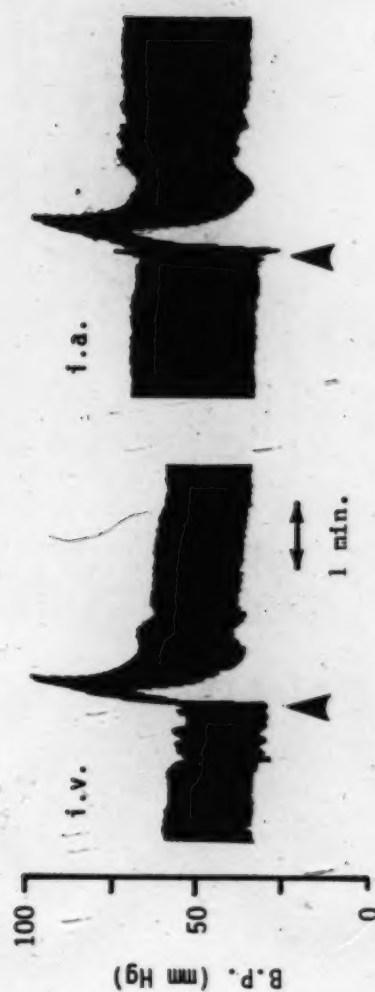


Figure 20. The effects of DMPP ($20 \mu\text{g/kg}$) injected i.v. or i.a., on B.P. in anesthetized paralyzed guinea pigs.

injected i.v.

4.6 Experiments with 5-H.T.

The aim was to determine whether 5-H.T.-induced bronchospasm has a reflex component that could be attributed to stimulation of C-fiber afferents in the lungs.

4.6.1 Effects on R and E

Injection of 5-H.T. (1.0–64.0 $\mu\text{g/kg}$), i.v. or i.a., induced dose-dependent increases in R (Fig. 21) and E (Fig. 22). Peak responses in R were significantly greater (4–5 times) when the same dose was injected i.v. than i.a. (Fig. 21), but differences were not significant for changes in E (see Fig. 22).

4.6.2 Cardiovascular effects

5-H.T. injected i.v. or i.a. produced a dose-dependent reductions in arterial blood pressure in most animals (20 out of 24). In the remaining animals, the blood pressure responses were either pressor or biphasic.

4.6.3 Effects of vagotomy and autonomic blockers

Bilateral vagotomy was without any effect on airway or cardiovascular responses induced by 5-H.T. given i.v. or i.a. Atropine (0.1 mg/kg), bethanidine (2.5 mg/kg), mecamlamine (2.5 mg/kg), pentolinium (1.0 mg/kg), and trimetaphan (2.5 mg/kg), had no significant effect on changes in R and E induced by 5-H.T. injected i.v. or i.a. (Table 11).

4.6.4 Effects of methysergide & SP-antagonists

Methysergide (0.1 mg/kg) completely abolished changes in R (see Fig. 21) and E (see Fig. 22), induced by i.v. or i.a. injection of 5-H.T. Neither [D-Pro²,D-Trp^{7,8}]-SP nor [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP had any significant effect on 5-H.T.-induced increases in R and E.

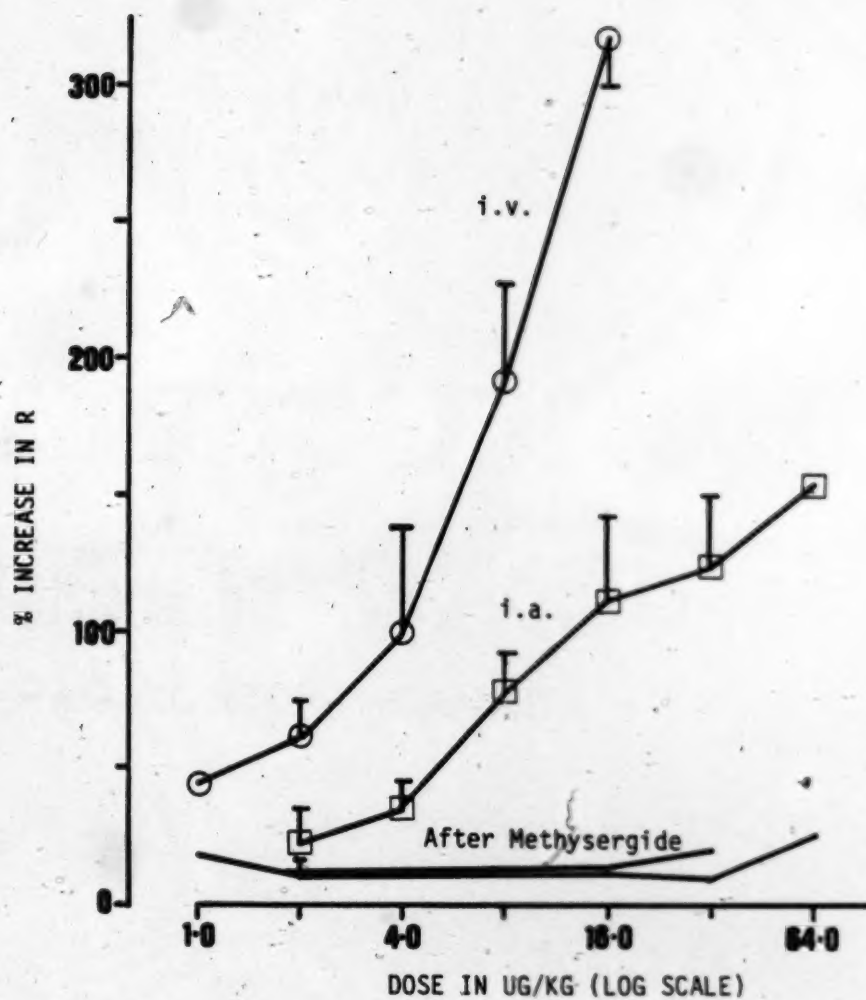


Figure 21. The effects of 5-H.T. on R before and after methysergide (0.1 mg/kg).

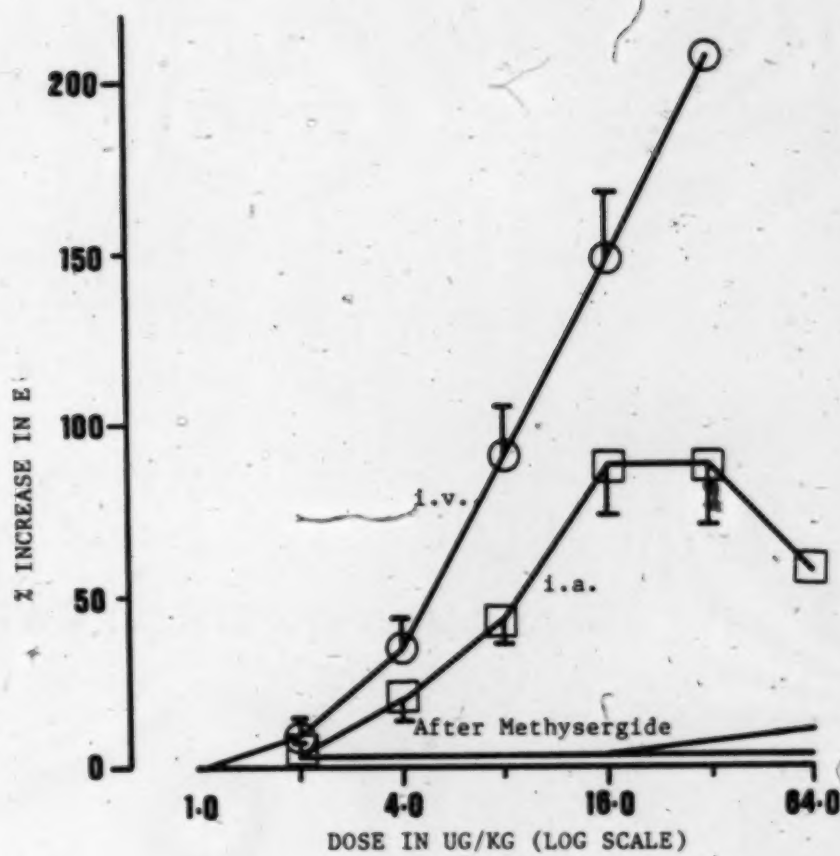


Figure 22.

The effects of 5-H.T. on E before and after methysergide (0.1 mg/kg).

Table 11: Effects of various autonomic blockers on per cent changes in airway resistance (R) and dynamic thoracic elastance (E), in response to 5-H.T., injected in the superior vena cava (i.v.) or intra-aortically (i.a.).

Blocker, Dose mg/kg	n	R (% increase)				E (% increase)			
		i.v.		i.a.		i.v.		i.a.	
		Control	Test	Control	Test	Control	Test	Control	Test
Atropine 0.1	4	49.4±17.9	79.7±34.8	74.6±8.2	75.6±20.4	90.2±12.4	81.5±10.6	68.0±20.1	26.9±8.4
Bethanidine 0.2	4	98.9±18.2	80.5±9.6	18.4±7.6	23.6±8.6	56.6±4.7	55.8±6.2	5.2±2.6	6.0±5.6
Mecamylamine 2.5	4	105.2±24.3	137.0±38.9	57.6±29.8	70.4±29.1	32.4±4.0	42.2±3.7	30.5±7.8	29.7±15.4
Trimetaphan 1.0	4	100.4±23.0	74.9±14.4	37.6±17.1	14.9±2.8	26.5±10.6	39.1±12.2	20.4±7.4	15.0±1.6
Pentolinium 1.0	4	80.0±10.6	105.1±21.8	100.7±36.3	149.6±16.2	73.9±8.9	86.0±13.4	55.7±9.1	67.2±6.8

4.7 Experiments with Capsaicin

Capsaicin was used to selectively stimulate C-fiber (especially J receptor) afferents in the lungs.

Injection of diluted vehicle alone (ethanol and Tween 80), i.v. or i.a., induced insignificant changes in baseline values of R and E.

4.7.1 Effects on R and E

Single doses of capsaicin (0.25–16 $\mu\text{g/kg}$) induced dose-dependent increases in R and E, whether given into the SVC, right atrium, or aortic arch (Fig. 23). The time course of such increases in R and E is shown in Figure 24. Dose-response curves were difficult to obtain: responses to a small single dose ($<2.0 \mu\text{g/kg}$) increased during repetitive dosing except when the interval between doses was prolonged, and larger doses ($>8.0 \mu\text{g/kg}$) induced tachyphylaxis. There was no qualitative difference in responses. However, peak responses were significantly greater after i.v. than after i.a. administration (Table 12); also, comparison of responses of similar magnitude (50–70% change) revealed significantly shorter intervals between injection of the drug and peak response for increases in R [i.v., $14.5 \pm 1.0 \text{ s}$, $n=24$; i.a., $21.0 \pm 1.5 \text{ s}$, $n=25$; $P < 0.001$] but not in E [i.v., $20.7 \pm 2.2 \text{ s}$, $n=24$; i.a., $26.4 \pm 2.5 \text{ s}$, $n=25$]. Capsaicin, given by either route, decreased the B.P. (Fig. 25); its effects on heart rate were not examined.

4.7.2 Effects of nerve sections and decentralization

Neither nerve sections nor decentralization significantly altered baseline R and E values. Capsaicin-induced changes in R and E were similar before and after decentralization, bilateral vagotomy, or glossopharyngealotomy (Table 13).

4.7.3 Effects of autonomic antagonists

The acute administration of mecamlamine, atropine, mepyramine, bethanidine, and sodium cromoglycate, or of baclofen, had no effect on capsaicin-induced changes in R and E

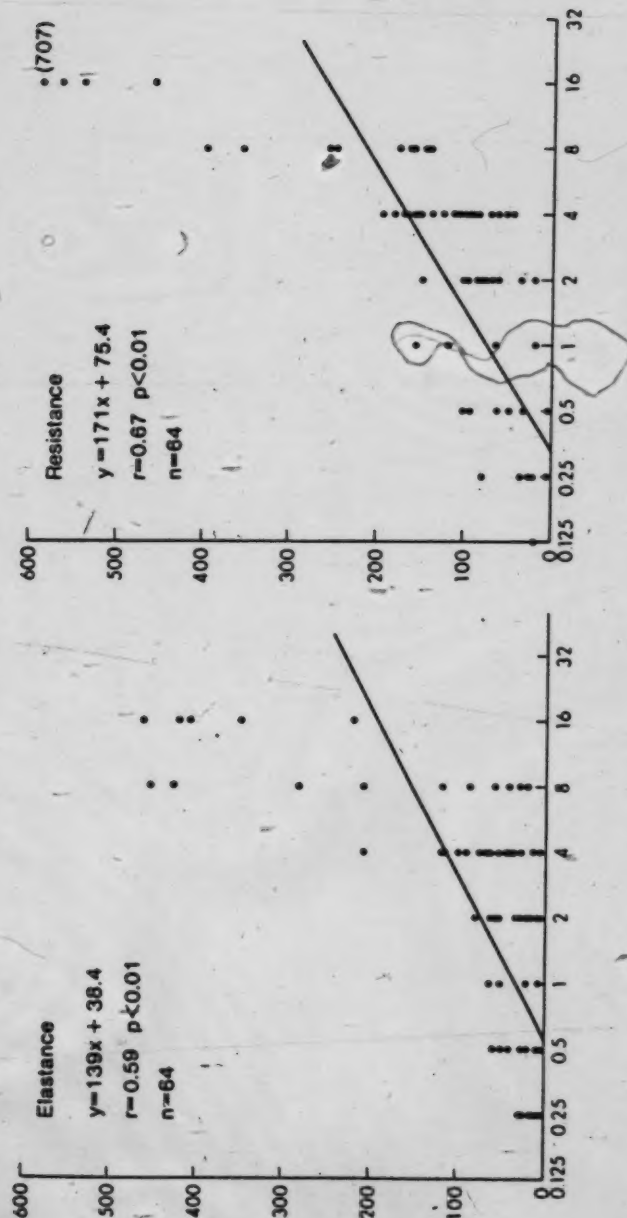


Figure 23. Scatter plots showing dose-response relationship between the amount of capsaicin injected i.v., in single doses, and observed increases in R and E. Ordinate: percentage increase from baseline; abscissa: dose $\mu\text{g}/\text{kg}$ (log scale).

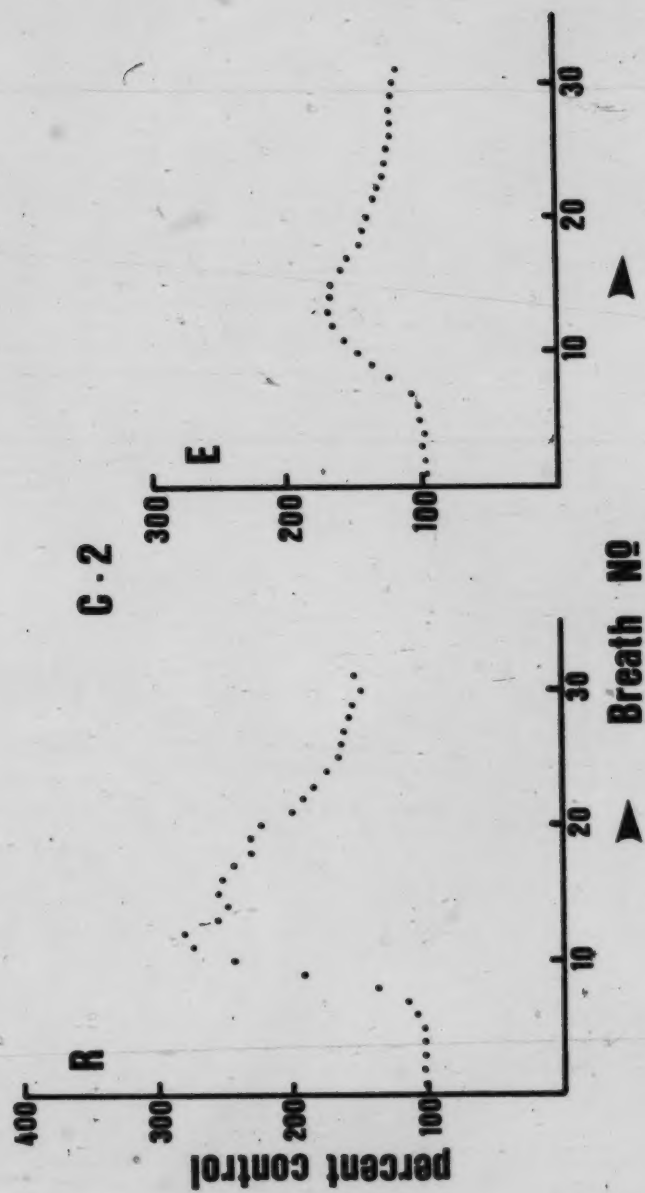


Figure 24. Breath-by-breath changes in R and E in response to capsaicin (2.0 $\mu\text{g/kg}$) injected i.v.

Table 12: Pulmonary flow resistance (R) and dynamic thoracic elastance (E) increases induced by increasing doses of capsaicin injected i.v. or i.a. Values are mean $\pm$ S.E. Figures in parentheses are numbers of experiments.

Capsaicin $\mu\text{g/kg}$	R (% increase)		E (% increase)	
	i.v.	i.a.	i.v.	i.a.
1	57.1 $\pm$ 8.3 (15)	10.1 $\pm$ 2.9† (12)	18.0 $\pm$ 4.6 (15)	6.1 $\pm$ 1.5† (12)
2	110.7 $\pm$ 16.2 (14)	20.5 $\pm$ 5.3† (15)	69.0 $\pm$ 14.8 (14)	35.6 $\pm$ 15.5† (15)
3	210.7 $\pm$ 21.4 (16)	31.0 $\pm$ 7.7† (16)	170.3 $\pm$ 39.0 (16)	48.0 $\pm$ 26.4† (16)

† P = <0.2; ‡ P = <0.01.



Figure 25.

The effects of capsaicin ($4.0 \mu\text{g/kg}$) injected i.v. on B.P. in an anesthetized paralyzed guinea pig.

Table 13: Effects of capsaicin, injected into the superior vena cava (i.v.) and intra-aortically (i.a.), before and after various nerve sections, and decentralization. The results are shown as percentage increase in pulmonary flow resistance (R) and dynamic thoracic elastance (E) from baseline. Values are mean $\pm$ S.E.

Treatment	Capsaicin $\mu\text{g/kg}$	n	i.v.				i.a.			
			R (% increase)		E (% increase)		R (% increase)		E (% increase)	
			Control	Test	Control	Test	Control	Test	Control	Test
Bilateral vagotomy	1.0	4	64.8 $\pm$ 14.5	63.2 $\pm$ 25.6	26.4 $\pm$ 4.5	34.2 $\pm$ 6.2	12.0 $\pm$ 8.4	20.0 $\pm$ 3.6	9.1 $\pm$ 4.8	8.2 $\pm$ 3.4
Bilateral glossopharyngealotomy	1.0	8	98.0 $\pm$ 20.8	96.3 $\pm$ 26.4	39.2 $\pm$ 8.4	40.4 $\pm$ 8.8	37.0 $\pm$ 13.4	26.3 $\pm$ 10.8	20.8 $\pm$ 10.8	14.4 $\pm$ 10.4
Decentralization	2.0	26†, 4	117.9 $\pm$ 31.7‡	113.0 $\pm$ 27.6	47.1 $\pm$ 16.7‡	42.6 $\pm$ 16.8	68.8 $\pm$ 5.4‡	87.6 $\pm$ 26.7	27.3 $\pm$ 5.7‡	2.9 $\pm$ 11.2

† Number of control experiments.

‡ Control values derived in previous experiments with the same dose of capsaicin.

(Table 14). Findings with mecamlamine and atropine after anesthesia with urethane, pentobarbital, or α -chloralose were similar (Table 15).

4.7.4 Effects of morphine and naloxone

Morphine (0.5 or 1.0 mg/kg) reduced the capsaicin-induced increases in R and E (Table 16). The effect was most marked on changes in R, but reached statistical significance only at the highest dose of capsaicin (8.0 μ g/kg). The inhibition was not reversed by naloxone, 0.05–0.1 mg/kg. Naloxone (0.1–0.8 mg/kg), by itself, had no effect on responses to capsaicin (Table 17).

4.7.5 Effects of capsaicin pretreatment

Capsaicin pretreatment (50 mg/kg, s.c.) made animals unresponsive to acute administration of this drug in the dose range 2.0–16.0 μ g/kg, and only minor responses were noted with a dose of 32.0 μ g/kg (Fig. 26).

4.7.6 Effects of antagonists to substance P

We examined the effects of two antagonists to SP on responses to capsaicin. All experiments were done after the administration of mepyramine and atropine; neither antagonist significantly altered baseline R and E values.

[D-Pro²,D-Trp^{7,9}]-SP (1.0 mg/kg) infused over 5 min had no effect on capsaicin-induced increases in R and E (Table 18). By contrast, this antagonist in doses of 2.0 mg/kg reduced capsaicin-induced increases in R or even converted them to decreases, i.e., bronchodilation ensued. However, this higher dose did not alter the increases in E. When this antagonist (2.0 mg/kg) was infused over 15 min and response to capsaicin was monitored from 30 min after the end of the infusion, the effects were similar to those after acute administration of this drug. Capsaicin-induced increases in R were significantly reduced but increases in E were unchanged.

Table 14: Effects of capsaicin, injected into the superior vena cava (i.v.) and intra-aortically (i.a.), before and after autonomic blockers. The results are shown as percentage increase in pulmonary-flow resistance (R) and dynamic thoracic elastance (E) from baseline. Values are means $\pm$ S.E.

Treatment mg/kg	Capsaicin μ g/kg	n	i.v.				i.a.			
			R (% increase)		E (% increase)		R (% increase)		E (% increase)	
			Control	Test	Control	Test	Control	Test	Control	Test
Mecamylamine 2	2.0	4	115.0 $\pm$ 19.3	153.9 $\pm$ 32.3	44.7 $\pm$ 10.9	39.1 $\pm$ 6.2	67.8 $\pm$ 14.9	74.8 $\pm$ 21.9	21.5 $\pm$ 11.1	29.6 $\pm$ 10.5
Atropine 0.1	4.0	4	200.0 $\pm$ 59.6	175.0 $\pm$ 55.6	96.1 $\pm$ 29.5	96.7 $\pm$ 24.2	—	—	—	—
Mepyramine 0.1	2.0	4	90.4 $\pm$ 14.4	112.1 $\pm$ 28.6	35.0 $\pm$ 12.3	38.2 $\pm$ 13.7	74.6 $\pm$ 21.4	84.3 $\pm$ 19.7	27.6 $\pm$ 7.7	24.8 $\pm$ 11.8
Bethanidine 2.5	2.0	4	141.6 $\pm$ 31.4	128.7 $\pm$ 19.4	37.8 $\pm$ 11.6	30.2 $\pm$ 12.7	—	—	—	—
Sodium cromoglycate 10	2.0	4	82.3 $\pm$ 27.3	70.6 $\pm$ 24.5	29.6 $\pm$ 8.9	31.2 $\pm$ 12.9	63.9 $\pm$ 18.5	78.4 $\pm$ 15.2	32.9 $\pm$ 15.2	26.7 $\pm$ 6.2
Baclofen 2.5	2.0	4	111.5 $\pm$ 25.9	108.4 $\pm$ 28.9	66.6 $\pm$ 18.0	66.6 $\pm$ 17.8	—	—	—	—

Table 15: Effects of capsaicin, injected into the superior vena cava (i.v.) in animals anesthetized with urethane, pentobarbital, or α -chloralose, before and after injection of mecamlamine or atropine. The results are shown as percentage increase in pulmonary-flow resistance (R) and dynamic thoracic elastance (E) from baseline. Values are mean $\pm$ S.E.

Anesthetic	Treatment	Capsaicin $\mu\text{g/kg}$	n	R (% increase)		E (% increase)	
				Control	Test	Control	Test
Urethane (1.5 mg/kg) i.p.	Mecamlamine 2.5 mg/kg	2.0	4	115.0 $\pm$ 19.3	153.9 $\pm$ 32.3	44.7 $\pm$ 10.9	39.1 $\pm$ 6.2
	Atropine 0.5 mg/kg	4.0	4	185.1 $\pm$ 59.4	120.4 $\pm$ 35.1	93.0 $\pm$ 21.1	59.4 $\pm$ 18.9
Pentobarbital (30-50 mg/kg) i.p.	Mecamlamine 2.5 mg/kg	4.0	4	243.6 $\pm$ 34.7	277.7 $\pm$ 51.2	157.5 $\pm$ 26.0	187.0 $\pm$ 34.9
	Atropine 0.5 mg/kg	4.0	4	229.6 $\pm$ 35.3	211.4 $\pm$ 21.3	230.6 $\pm$ 12.9	211.4 $\pm$ 30.3
α -Chloralose (80-100 mg/kg) i.p.	Mecamlamine 2.5 mg/kg	4.0	4	198.6 $\pm$ 16.4	214.7 $\pm$ 20.2	153.5 $\pm$ 41.0	195.5 $\pm$ 41.8
	Atropine 0.5 mg/kg	4.0	4	182.3 $\pm$ 27.8	184.4 $\pm$ 40.6	237.9 $\pm$ 27.6	215.0 $\pm$ 39.3

Table 16: Pulmonary flow resistance (R) and dynamic thoracic elastance (E) increases induced by various doses of capsaicin (i.v.) before and after injection of morphine, and the effects of naloxone on capsaicin-induced increases after morphine. Results are shown as percentage increases in R and E over baseline. Values are mean $\pm$ S.E.

Capsaicin $\mu\text{g/kg}$	n	Control		Morphine 0.5 mg/kg		Morphine 1.0 mg/kg		Morphine 1.0 mg/kg		Naloxone 5 $\mu\text{g/kg}$	
		R	E	R	E	R	E	R	E	R	E
1.0	3	7.9 $\pm$ 1.5	3.4 $\pm$ 1.3	13.4 $\pm$ 8.9	11.2 $\pm$ 8.6	5.8 $\pm$ 0.1	1.7 $\pm$ 0.3	—	—	—	—
2.0	4	3.9 $\pm$ 12.6	12.6 $\pm$ 4.5	22.5 $\pm$ 9.0	12.9 $\pm$ 9.3	16.9 $\pm$ 4.8	7.4 $\pm$ 4.0	14.3 $\pm$ 4.4	7.6 $\pm$ 5.6	11.3 $\pm$ 2.4	4.2 $\pm$ 1.7
4.0	4	100.4 $\pm$ 21.9	50.7 $\pm$ 26.3	67.2 $\pm$ 21.4	95.5 $\pm$ 21.2	56.0 $\pm$ 14.8	34.3 $\pm$ 12.1	41.4 $\pm$ 4.2	32.5 $\pm$ 24.0	47.4 $\pm$ 22.7	28.1 $\pm$ 22.4
8.0	4	221.4 $\pm$ 20.1	189.1 $\pm$ 48.1	134.9 $\pm$ 24.3†	98.4 $\pm$ 15.0	119.3 $\pm$ 26.4†	113.9 $\pm$ 29.9	121.4 $\pm$ 37.2	131.3 $\pm$ 34.4	99.3 $\pm$ 35.9	112.0 $\pm$ 22.9

†P = <0.05

Table 17: Effects of naloxone on capsaicin-induced increases in pulmonary-flow resistance (R) and dynamic thoracic elastance (E). Dose of capsaicin: 2 μ g/kg i.v. Results are shown as percentage increase in R and E from baseline. Values are means $\pm$ S.E.

Naloxone mg/kg	n	R (% increase)		E (% increase)	
		Control	Test	Control	Test
0.1	6	166.8 $\pm$ 41.8	116.6 $\pm$ 26.1	68.8 $\pm$ 26.7	59.4 $\pm$ 20.4
0.2	4	183.5 $\pm$ 39.7	107.2 $\pm$ 34.5	33.2 $\pm$ 11.7	14.2 $\pm$ 5.3
0.4	4	159.2 $\pm$ 45.8	90.8 $\pm$ 19.9	51.5 $\pm$ 20.3	27.3 $\pm$ 7.7
0.8	4	186.6 $\pm$ 58.7	172.5 $\pm$ 50.2	69.0 $\pm$ 43.4	77.9 $\pm$ 25.8

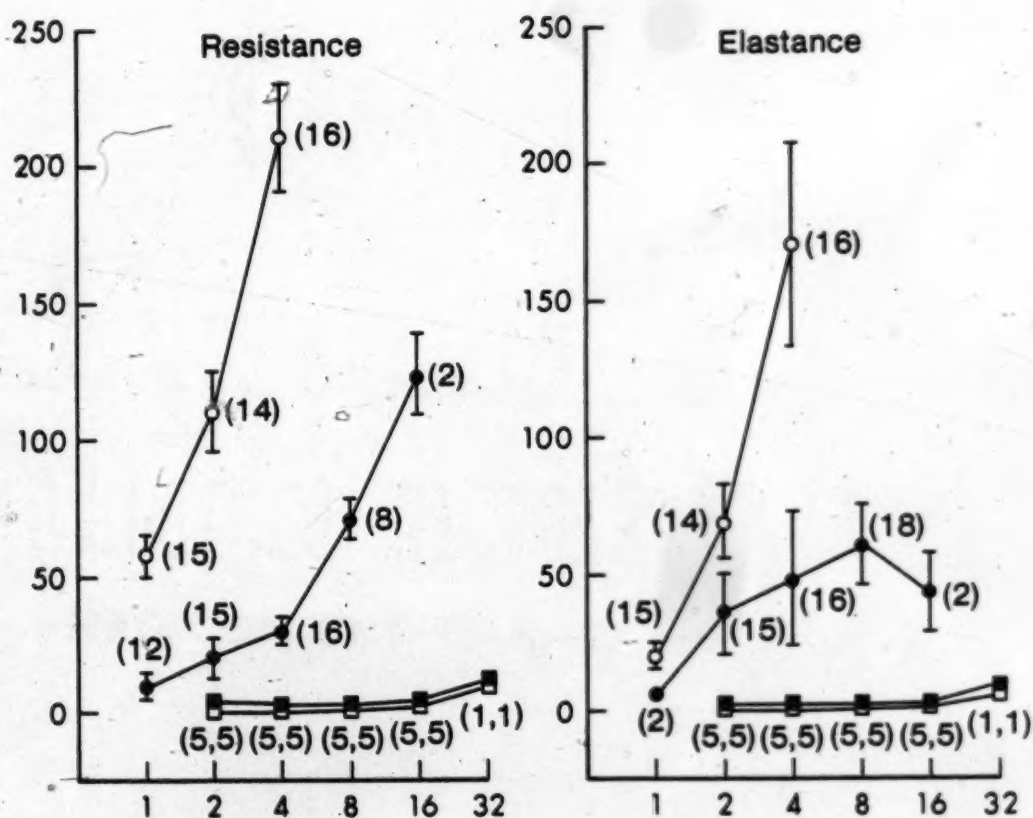


Figure 26.

Increases in R and E in response to capsaicin injected i.v. (open symbols) and i.a. (filled symbols) without capsaicin pretreatment (circles) and 7 days after s.c. administration of the drug, 50 mg/kg (squares). Values are mean $\pm$ S.E. Ordinate: percentage increase from baseline; abscissa: dose μ g/kg (log scale).

Table 18: Percentage increases from baseline in pulmonary-flow resistance (R) and dynamic thoracic elastance (E) induced by capsaicin (CAP) before and after i.v. infusion of [D-Pro², D-Trp⁷, ⁹]-SP (PTSP), or [D-Arg¹, D-Pro², D-Trp⁷, ⁹, Leu¹¹]-SP (ALSP). All animals received mepyramine and atropine. Values are means $\pm$ S.E.

Treatment mg/kg	Infusion time min	Agonist μ g/kg	n	R (% increase)		E (% increase)	
				Control	Test	Control	Test
PTSP, 1.0	5	CAP 1.0	5	119.8 $\pm$ 24.6	50.2 $\pm$ 7.9	48.0 $\pm$ 14.4	44.0 $\pm$ 12.8
PTSP, 2.0	5	CAP 2.0	4	122.0 $\pm$ 12.3	4.7 $\pm$ 2.6†	61.0 $\pm$ 22.4	47.6 $\pm$ 13.9
PTSP, 2.0	15	CAP 2.0	5	153.9 $\pm$ 34.8	47.9 $\pm$ 20.4†	96.0 $\pm$ 33.0	51.6 $\pm$ 10.2
ALSP, 1.0 ¹	15	CAP 2.0	4	147.4 $\pm$ 20.8	62.2 $\pm$ 19.1†	58.3 $\pm$ 16.9	30.3 $\pm$ 10.9

† P = <0.05

¹0.3 mg/kg was also injected before each dose of agonist (see Lundberg et al., 1983a).

Infusion of [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP (1.0 mg/kg), with 0.3 mg/kg injected before each agonist drug, significantly reduced capsaicin-induced increases in R but had no effect on increases in E induced by capsaicin (Table 18).

4.8 Experiments with Tachykinins

Involvement of tachykinins in capsaicin-induced bronchospasm is suggested by above experiments. Thus, we performed experiments with selected tachykinins to analyze their effects on airways and to define the specificity of two antagonists to SP against these tachykinins.

4.8.1 Effects on R and E

SP (2.0–4.0 μ g/kg), physalaemin (PH) (0.5–1.0 μ g/kg), and eledoisin-related peptide (ERP) (0.5–2.0 μ g/kg) induced dose-dependent increases in R (Figs 27 & 28) and E (Figs 29 & 30), that were about 4 times greater after i.v. than i.a. injection. By either route, PH was about 5 times and ERP about twice as potent as SP. PH-induced increases in R and E took significantly longer ($P < 0.05$) to reach maximum than similar increases due to SP or ERP; for each tachykinin, the peak response time was similar for the same dose given i.v. and i.a. (Table 19). Injection of tachykinins into the descending aorta (2 experiments) did not increase R and E.

4.8.2 Cardiovascular effects

All tachykinins, whether injected i.v. or i.a., induced hypotension that was maximal within 60 s and of similar magnitude whether the same dose was given i.v. or i.a. PH-induced hypotension was of longest duration (Fig. 31). The blood pressure always returned to baseline values within 15 min after the injection of tachykinin. Injections into the descending aorta induced depressor responses similar to those after i.v. or i.a. injection.

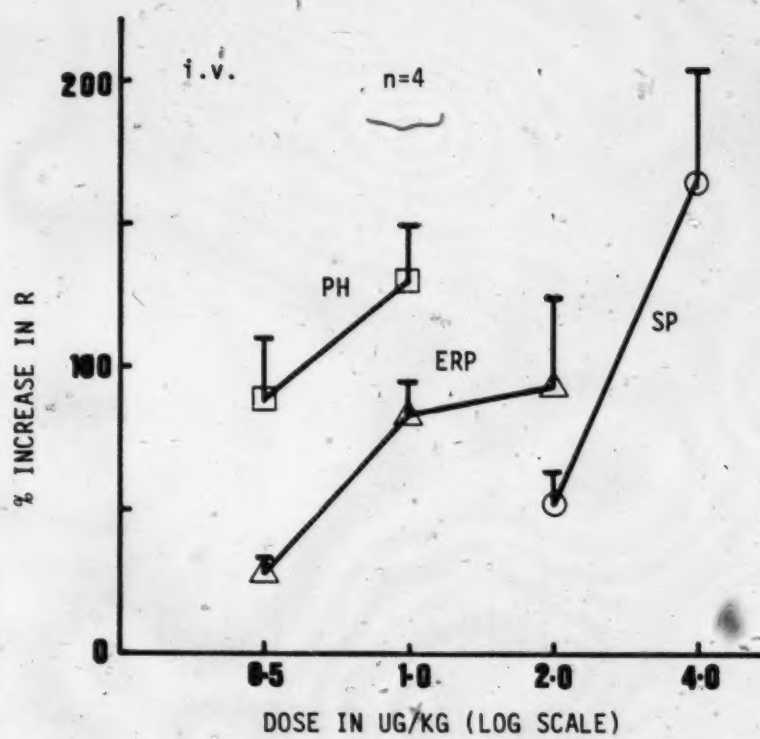


Figure 27. The effects of SP, ERP, and PH, injected i.v. on R.

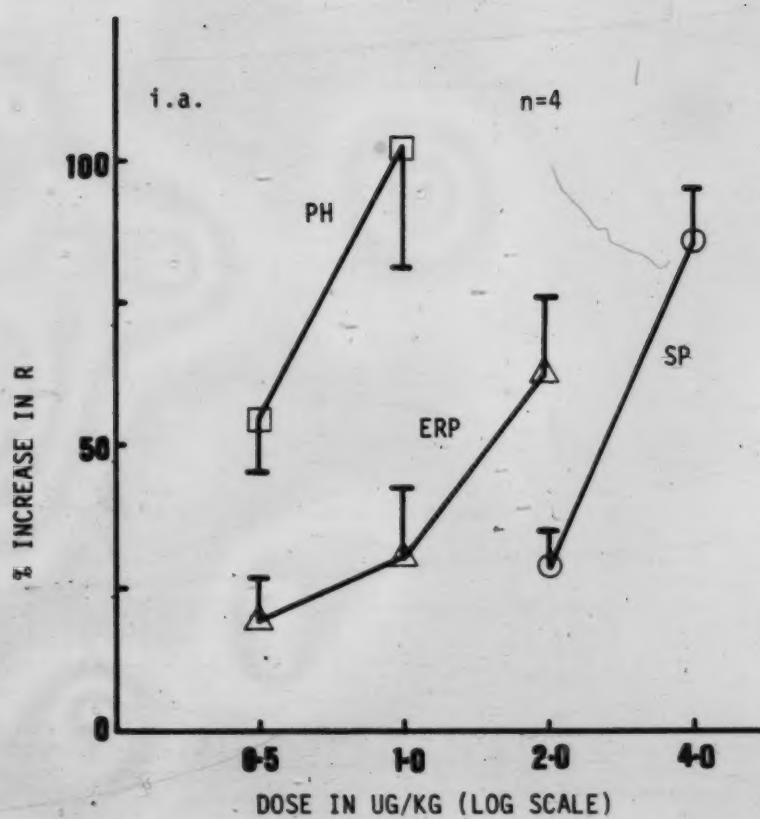


Figure 28. The effects of SP, ERP, and PH, injected i.a. on R.

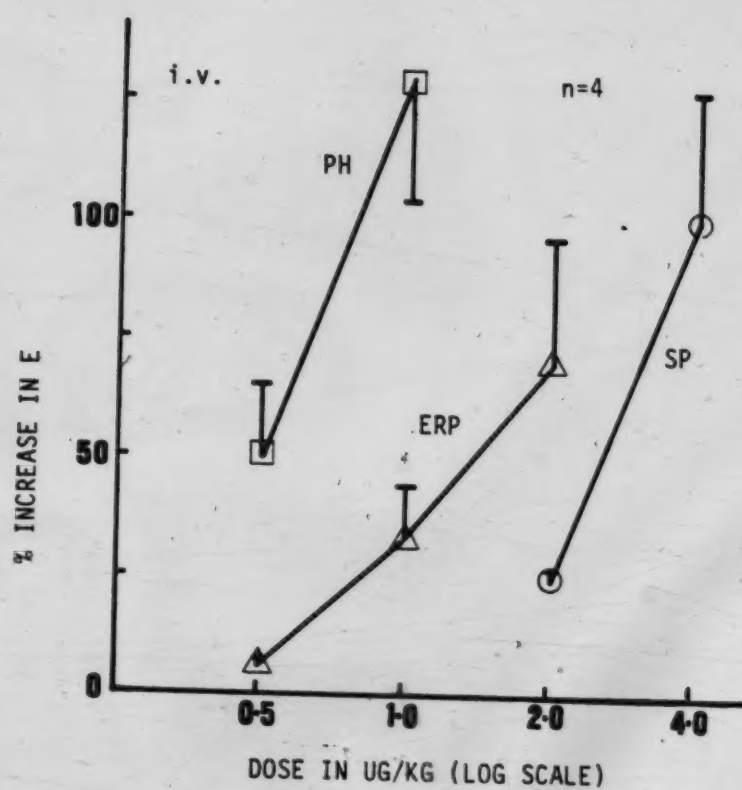


Figure 29. The effects of SP, ERP, and PH, injected i.v. on E.

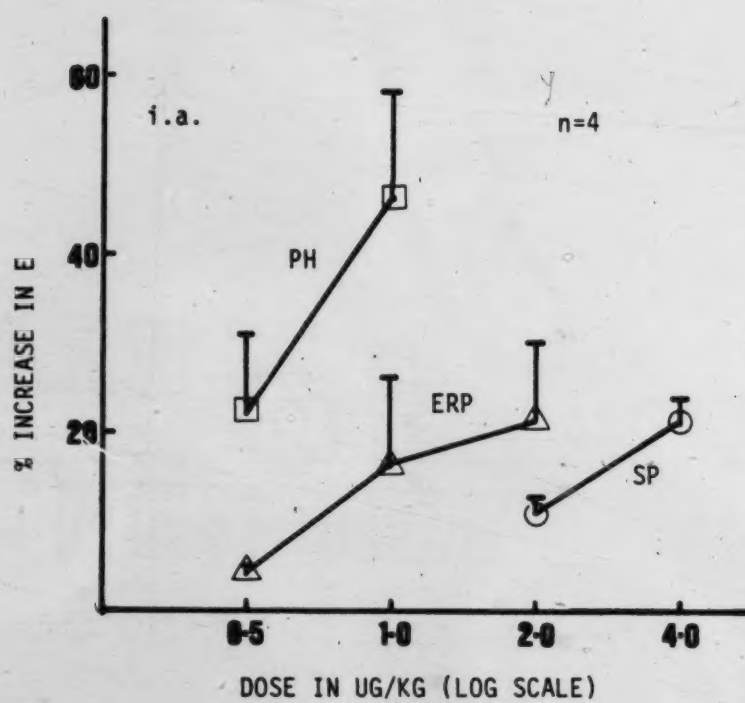


Figure 30. The effects of SP, ERP, and PH, injected i.a. on E.

Table 19: Time (in seconds) to maximum increases in pulmonary-flow resistance (R) and dynamic thoracic elastance (E), in response to tachykinins injected intravenously (i.v.) and intra-aortically (i.a.). Values are means $\pm$ S.E. Number in parentheses indicates the number of experiments.

Agonist	Dose $\mu\text{g/kg}$	R		E	
		i.v.	i.a.	i.v.	i.a.
SP	2.0	17.2 $\pm$ 1.0 (5)	20.5 $\pm$ 4.4 (4)	23.6 $\pm$ 2.0 (5)	26.9 $\pm$ 5.0 (4)
	4.0	23.6 $\pm$ 1.6 (5)	32.7 $\pm$ 2.6 $\ddagger$ (4)	24.2 $\pm$ 5.9 (5)	32.7 $\pm$ 3.6 (4)
ERP	0.5	22.8 $\pm$ 1.0 (2)	27.8 $\pm$ 6.0 (2)	24.8 $\pm$ 1.0 (2)	29.7 $\pm$ 0.0 $\dagger$ (2)
	1.0	19.9 $\pm$ 1.7 (5)	26.8 $\pm$ 4.4 (4)	19.1 $\pm$ 2.5 (5)	19.9 $\pm$ 3.6 (4)
	2.0	17.8 $\pm$ 1.4 $\dagger$ (4)	29.7 $\pm$ 1.1 $\ddagger$ (3)	21.3 $\pm$ 3.7 (4)	25.1 $\pm$ 1.7 (3)
PH	0.25	29.7 $\pm$ 2.0 (2)	26.8 $\pm$ 10.2 (2)	43.6 $\pm$ 7.9 (2)	36.7 $\pm$ 3.0 (2)
	0.5	32.5 $\pm$ 4.9 (5)	33.6 $\pm$ 4.3 (4)	44.3 $\pm$ 8.1 (5)	37.1 $\pm$ 5.0 (4)
	1.0	34.4 $\pm$ 1.8 (3)	33.7 $\pm$ 4.1 (3)	33.1 $\pm$ 3.6 (3)	39.0 $\pm$ 0.7 (3)

SP: substance P; ERP: eledoisin-related peptide; PH: physalaemin.

$\dagger$ P = <0.05 ; $\ddagger$ P = <0.01 .

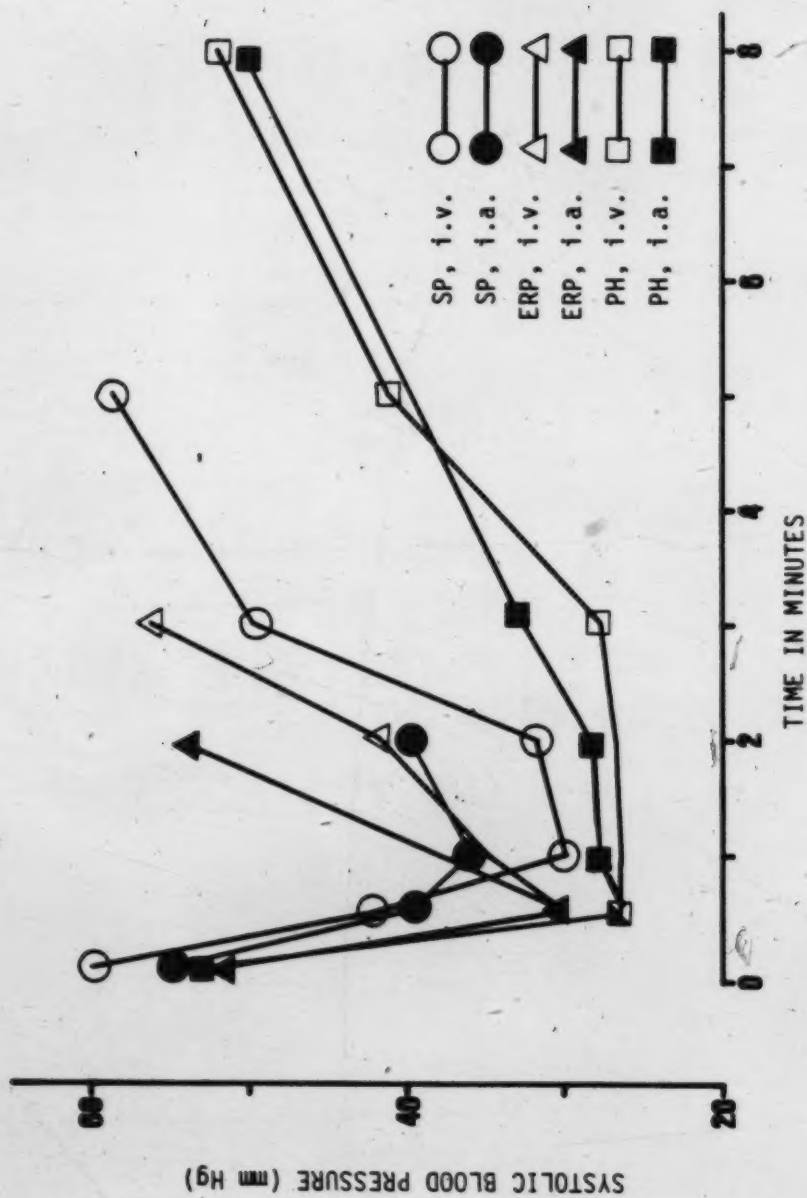


Figure 31. A typical experiment showing the effects of SP, ERP, and PH on B.P.

4.8.3 Effects of antagonists and nerve sections

Tachykinin-induced increases in R and E, and decreases in MABP, were unaltered by bilateral vagotomy (Table 20). Similarly, atropine (0.5 mg/kg), mepyramine (0.5 mg/kg), and mecamlamine (2.5 mg/kg) had no effect on tachykinin-induced increases in R and E (Table 21), but depressor responses to all tachykinins were reduced after mecamlamine.

4.8.4 Effects of morphine

Morphine reduced PH-induced increases in R and E without altering those due to SP and ERP (Table 22). The inhibition was not reversed by naloxone in doses of 0.01 and 0.1 mg/kg. Morphine had no effect on depressor responses to any of the tachykinins examined.

4.8.5 Effects of capsaicin pretreatment

SP- and PH-induced increases in E were significantly smaller in capsaicin-treated animals than in untreated controls, but SP- and PH-induced increases in R and ERP-induced increases in R and E were not altered (Table 23).

4.8.6 Effects of slow infusion

Infusion of ERP and PH for 30 min did not alter the bronchoconstrictor or depressor responses to acute injection of SP, PH, or ERP; nor was tachyphylaxis observed for either bronchospastic or depressor responses.

4.8.7 Effects of antagonists to substance P

[D-Pro²,D-Trp^{7,8}]-SP (1.0 or 2.0 mg/kg) had no effect on SP- or PH-induced increases in R and E, but significantly reduced ERP-induced increases (Figs 32 & 33). It reduced the depressor responses to all tachykinins tested.

[D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP significantly reduced increases in R and E induced by SP and PH, but not ERP (Figs 34 & 35). The depressor effects of all three tachykinins were reduced by this antagonist.

Table 20: Effects of bilateral vagotomy on tachykinin-induced percentage increases in pulmonary-flow resistance (R) and dynamic thoracic elastance (E). Results expressed as means $\pm$ S.E.

Tachykinin Dose, μ g/kg	n	R (% increase)		E (% increase)	
		Control	Test	Control	Test
SP, 1.0	4	43.3 $\pm$ 9.4	43.1 $\pm$ 12.6	13.0 $\pm$ 4.0	28.7 $\pm$ 9.7
SP, 2.0	4	115.7 $\pm$ 30.9	96.3 $\pm$ 23.3	36.2 $\pm$ 9.5	42.1 $\pm$ 23.0
SP, 4.0	4	217.7 $\pm$ 18.2	221.5 $\pm$ 53.2	114.4 $\pm$ 23.2	97.1 $\pm$ 34.2
ERP, 1.0	4	141.2 $\pm$ 31.8	129.6 $\pm$ 32.4	65.7 $\pm$ 21.1	57.4 $\pm$ 13.7
PH, 0.5	4	164.8 $\pm$ 29.6	172.6 $\pm$ 41.1	81.3 $\pm$ 25.7	78.6 $\pm$ 18.2

SP: substance P; ERP: eledoisin-related peptide; PH: physalaemin.

Table 21: Effects of substance P (SP), elodisin-related peptide (ERP), and physalaemin (PH), injected i.v., before and after autonomic blockers. The results are shown as per cent increase in pulmonary-flow resistance (R) and dynamic thoracic elastance (E) from baseline. Values are mean $\pm$ S.E.

Agonist (Dose μ g/kg)	Treatment (Dose mg/kg)	n	R (% increase)		E (% increase)	
			Control	Test	Control	Test
SP (2.0)	Atropine sulfate (0.5)	6	135.0 $\pm$ 21.2	138.8 $\pm$ 16.5	43.4 $\pm$ 13.1	40.2 $\pm$ 12.3
ERP (1.0)	Atropine sulfate (0.5)	4	85.3 $\pm$ 29.4	78.6 $\pm$ 31.3	33.2 $\pm$ 16.3	32.3 $\pm$ 16.1
PH (0.5)	Atropine sulfate (0.5)	4	163.5 $\pm$ 28.6	125.1 $\pm$ 63.1	55.6 $\pm$ 30.2	72.8 $\pm$ 38.7
SP (2.0)	Mepyramine (0.5)	4	83.5 $\pm$ 18.1	75.4 $\pm$ 22.7	39.4 $\pm$ 12.2	40.8 $\pm$ 18.8
ERP (1.0)	Mepyramine (0.5)	4	90.2 $\pm$ 24.8	86.3 $\pm$ 22.2	35.9 $\pm$ 13.0	37.6 $\pm$ 14.1
PH (0.5)	Mepyramine (0.5)	4	121.1 $\pm$ 20.9	135.8 $\pm$ 67.5	52.0 $\pm$ 18.3	50.7 $\pm$ 18.7
SP (2.0)	Mecamylamine (2.5)	4	128.3 $\pm$ 19.9	160.4 $\pm$ 53.4	38.9 $\pm$ 7.0	54.2 $\pm$ 13.8
ERP (1.0)	Mecamylamine (2.5)	4	115.3 $\pm$ 46.6	146.1 $\pm$ 45.9	35.3 $\pm$ 9.7	54.5 $\pm$ 34.2
PH (0.5)	Mecamylamine (2.5)	4	154.9 $\pm$ 63.9	185.8 $\pm$ 58.8	62.3 $\pm$ 11.8	73.4 $\pm$ 18.6

SP: substance P; ERP: elodisin-related peptide; PH: physalaemin.

Table 22: Effects of morphine on tachykinin-induced increases in pulmonary-flow resistance (R) and dynamic thoracic elastance (E). SP, substance P; ERP, eledoisin-related peptide; PH, physalaemin.

Tachykinin $\mu\text{g/kg}$	n	R (% increase)		E (% increase)	
		Control	Test	Control	Test
SP, 2.0	4	173.2 $\pm$ 31.3	203.2 $\pm$ 23.0	67.7 $\pm$ 22.2	66.3 $\pm$ 9.0
ERP, 1.0	4	152.0 $\pm$ 41.4	151.4 $\pm$ 7.2	63.6 $\pm$ 17.8	75.1 $\pm$ 16.1
PH, 0.5	4	226.8 $\pm$ 25.0	170.4 $\pm$ 12.0 $\ddagger$	100.4 $\pm$ 40.0	66.3 $\pm$ 22.1 $\dagger$

$\dagger P = <0.1$; $\ddagger P = <0.05$

Table 23: Tachykinin-induced increases in pulmonary-flow resistance (R) and dynamic thoracic elastance (E) in capsaicin-pretreated (50 mg/kg, s.c.) and untreated animals. Results expressed as mean $\pm$ S.E. SP, substance P; ERP, eledoisin-related peptide; PH, physalaemin.

Tachykinin $\mu\text{g/kg}$	n	R (% increase)		E (% increase)	
		Untreated	Treated	Untreated	Treated
SP 4.0	6	170.1 $\pm$ 20.8	116.2 $\pm$ 28.8	101.1 $\pm$ 31.2	30.7 $\pm$ 15.4†
ERP 2.0	4	122.9 $\pm$ 35.6	110.7 $\pm$ 15.9	70.0 $\pm$ 26.6	57.1 $\pm$ 11.8
PH 1.0	4	205.0 $\pm$ 64.8	123.3 $\pm$ 46.0	130.0 $\pm$ 34.9	45.1 $\pm$ 19.6‡

† P = < 0.05; ‡ P = < 0.01

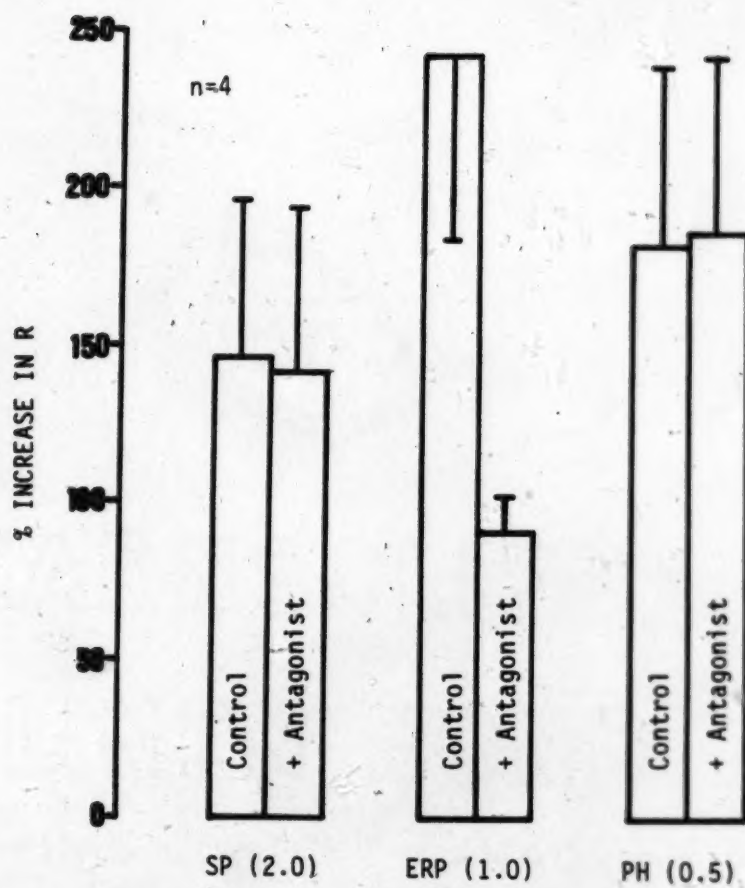


Figure 32. The effects of [D-Pro¹,D-Trp⁷]-SP on increases in R induced by SP, ERP, or PH. Numbers in parentheses indicate the dose of tachykinin in μg/kg.

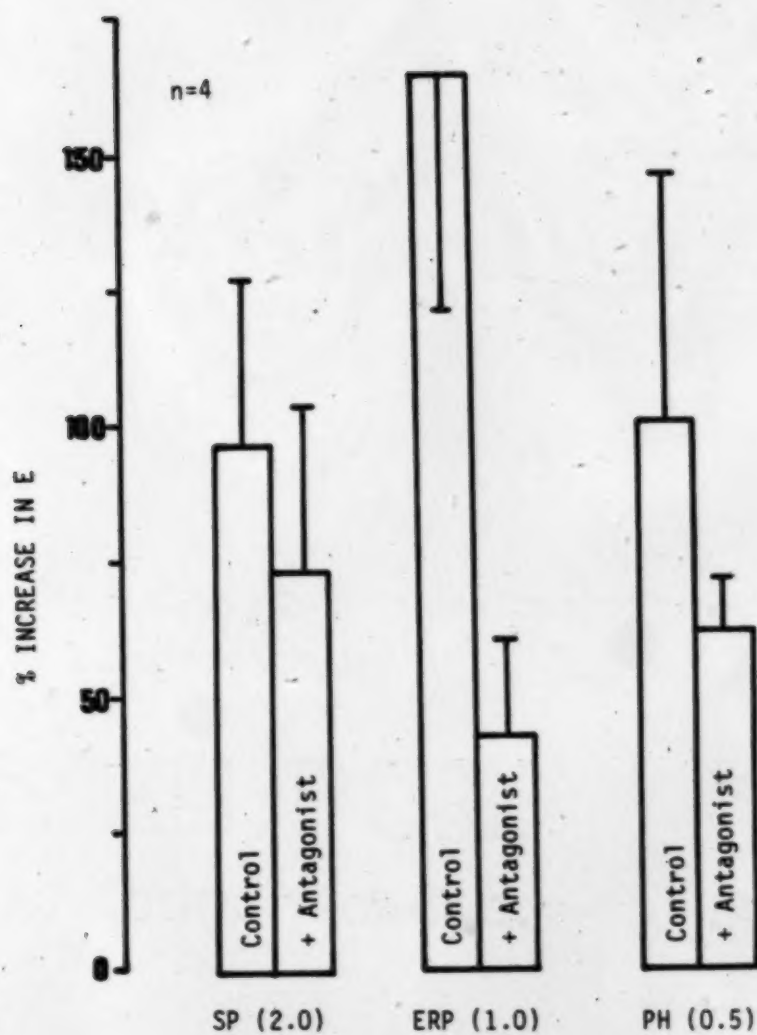


Figure 33.

The effects of [D-Pro³,D-Trp^{7,7}]-SP on increases in E induced by SP, ERP, or PH. Numbers in parentheses indicate the dose of tachykinin in μg/kg.

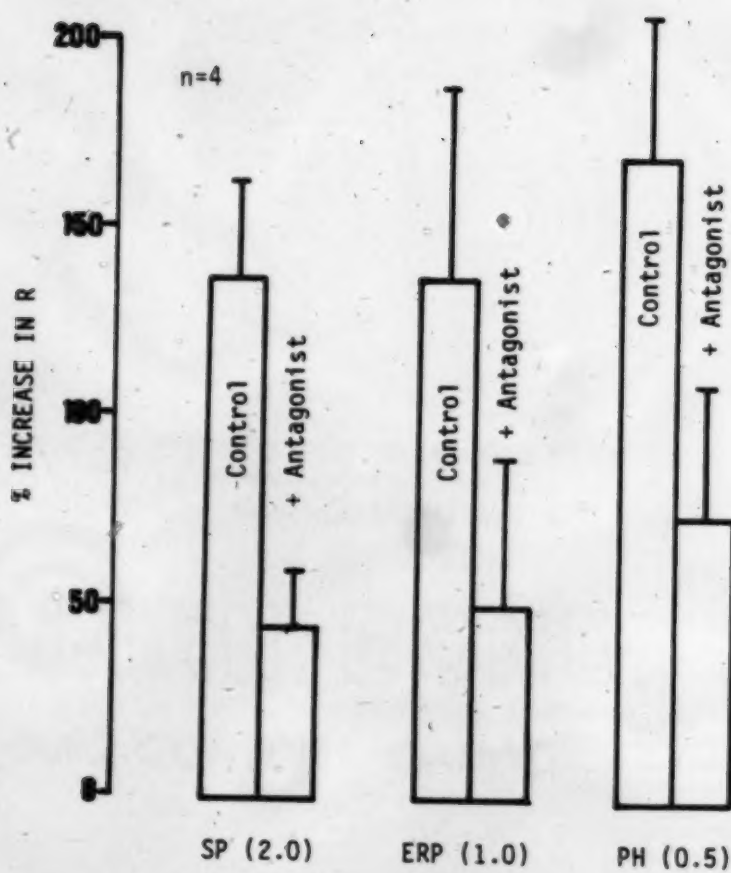


Figure 34.

The effects of $[D\text{-Arg}^1, D\text{-Pro}^2, D\text{-Trp}^{10}, \text{Leu}^{11}]$ -SP (1.0+0.3 mg/kg) on increases in R induced by SP, ERP, or PH. Numbers in parentheses indicate the dose of tachykinin in $\mu\text{g/kg}$.

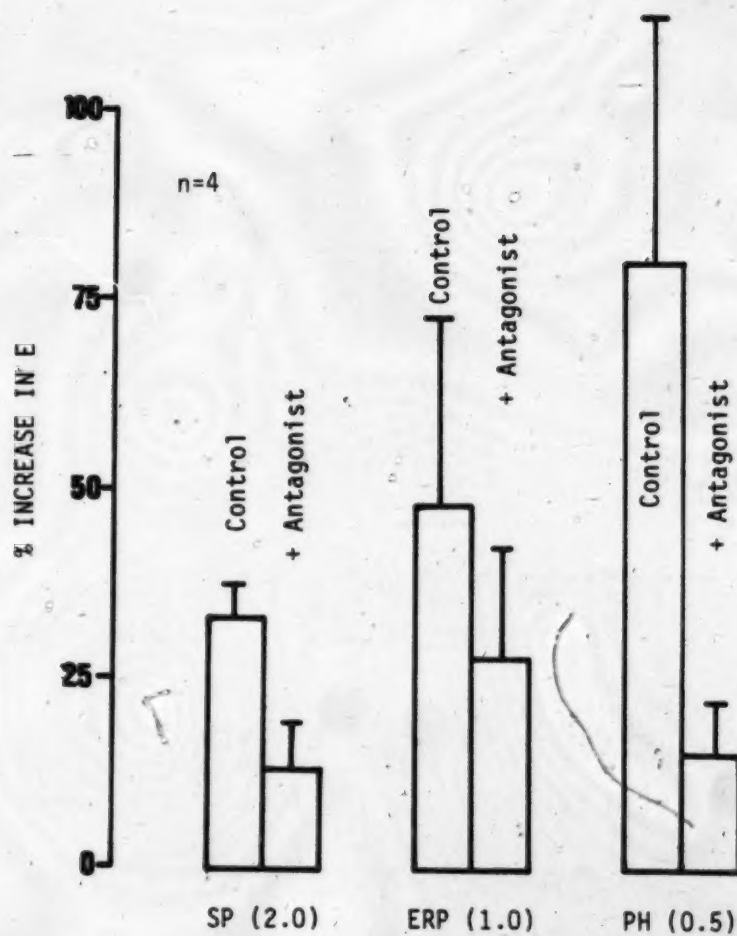


Figure 35.

The effects of $[\text{D-Arg}^1, \text{D-Pro}^1, \text{D-Trp}^9, \text{Leu}^{11}]$ -SP (1.0+0.3 mg/kg) on increases in E induced by SP, ERP, or PH. Numbers in parentheses indicate the dose of tachykinin in $\mu\text{g/kg}$.

4.9 Experiments with vagal stimulation

The aim was to determine whether tachykinins are released from vagal afferents in response to electrical stimulation of the vagus.

4.9.1 Airway responses

4.9.1.1 Central stimulation

Central stimulation of either vagus nerve (other intact) did not alter baseline values of R and E, or change MABP.

4.9.1.2 Peripheral stimulation

Unilateral stimulation caused small increases in R and E, and a fall in MABP. These responses were similar, whether the contralateral nerve was intact or had been sectioned. Bilateral stimulation induced significantly greater percent increases in R [bilateral, 153.0 ± 18.5 , $n=18$; unilateral, 27.8 ± 2.8 , $n=18$; $P < 0.001$] and E [bilateral, 99.5 ± 11.6 , $n=18$; unilateral, 52.1 ± 6.7 , $n=18$; $P < 0.01$] than unilateral stimulation. Increases in R and E after unilateral and bilateral stimulation were pulse-duration (Fig. 36) and pulse-frequency dependent (Fig. 37). Breath-by-breath changes in R and E in response to nerve stimulation are shown in Figures 38 & 39. The increases in E lasted longer than increases in R. However elastance always returned to baseline values within a period of 10 min. All results reported below are with bilateral nerve stimulation. Comparison of responses of similar magnitude (50–70% change) after vagal stimulation revealed that time to peak response for R [16.8 ± 0.8 s, $n=12$] was significantly less ($P < 0.001$) than time to peak response for E [22.2 ± 0.6 s, $n=17$].

4.9.2 Effects of atropine

Atropine (0.1 mg/kg) abolished increases in R and E induced by submaximal (0.1–0.5 ms) vagal stimulation (Figs 40 & 41), but it failed to abolish increases in R and E induced by maximal (5 ms) stimulation (Figs 40 & 41). As in untreated controls, the time taken to reach

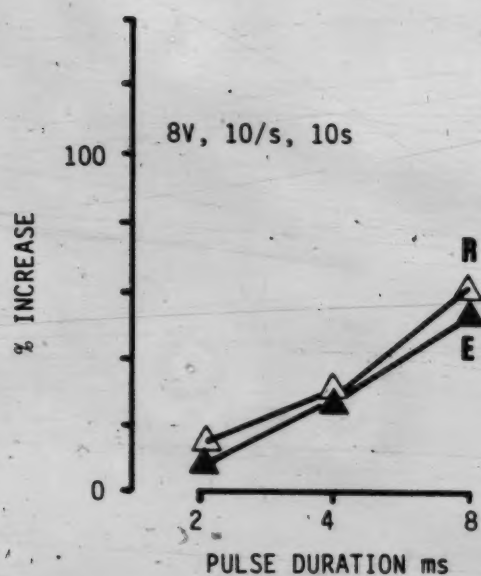


Figure 36.

A typical experiment showing relationship between pulse duration and the increases in R and E in response to vagal stimulation.

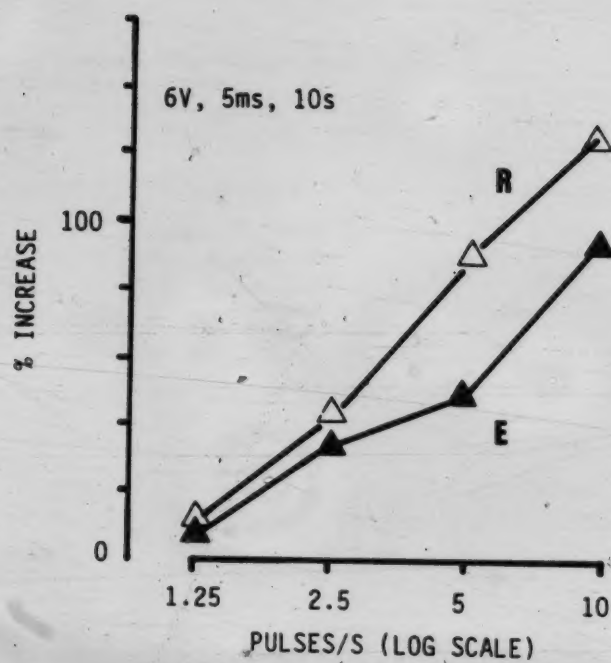


Figure 37.

A typical experiment showing relationship between pulse frequency and the increases in R and E in response to vagal stimulation.

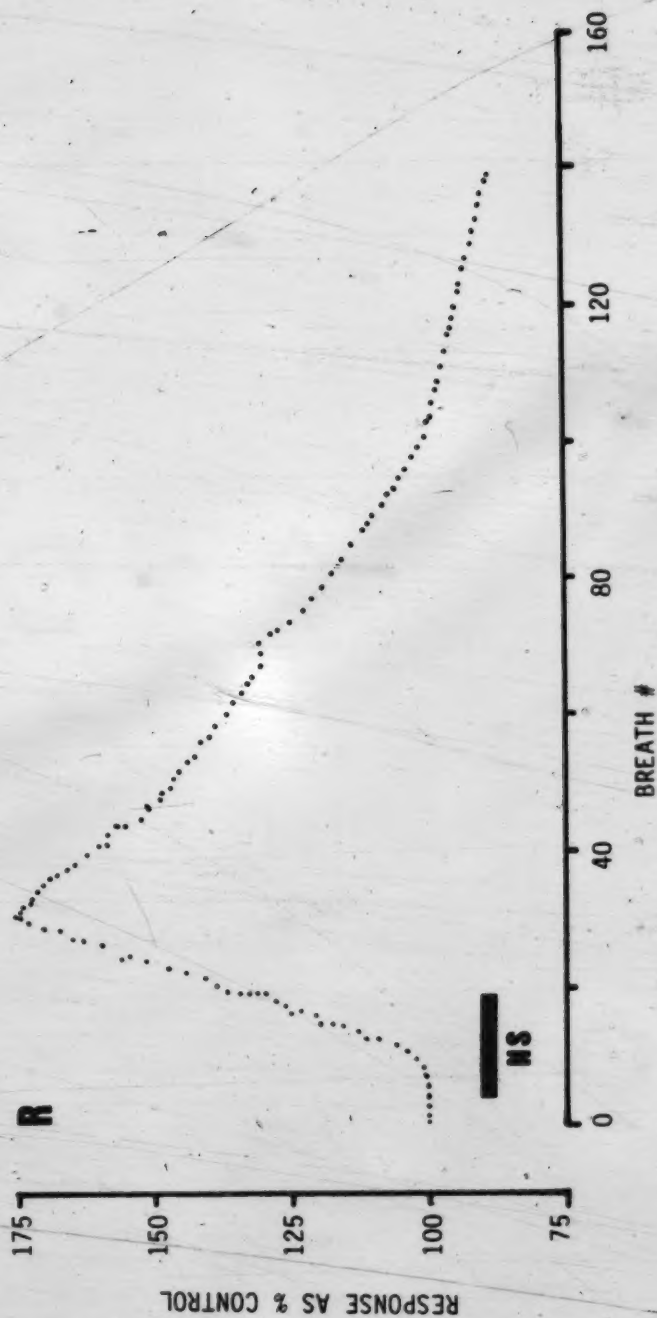


Figure 38. Breath-by-breath changes in R in response to bilateral vagal stimulation.

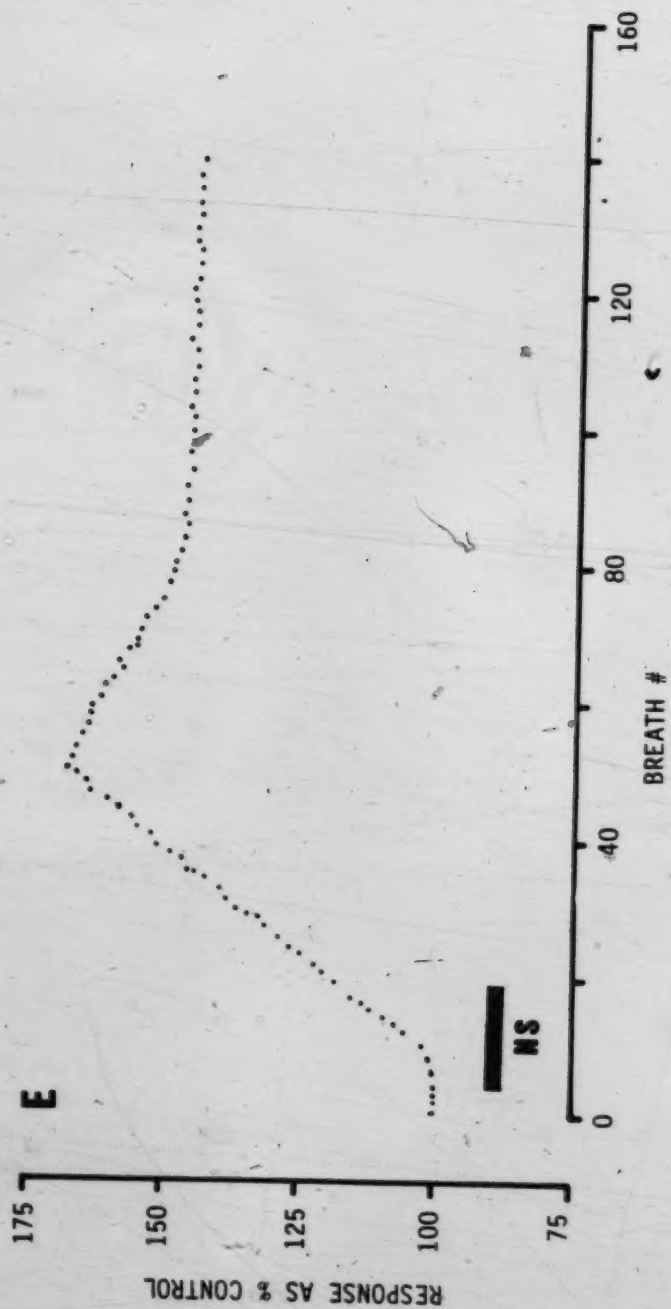


Figure 39. Breath-by-breath changes in E in response to bilateral vagal stimulation.

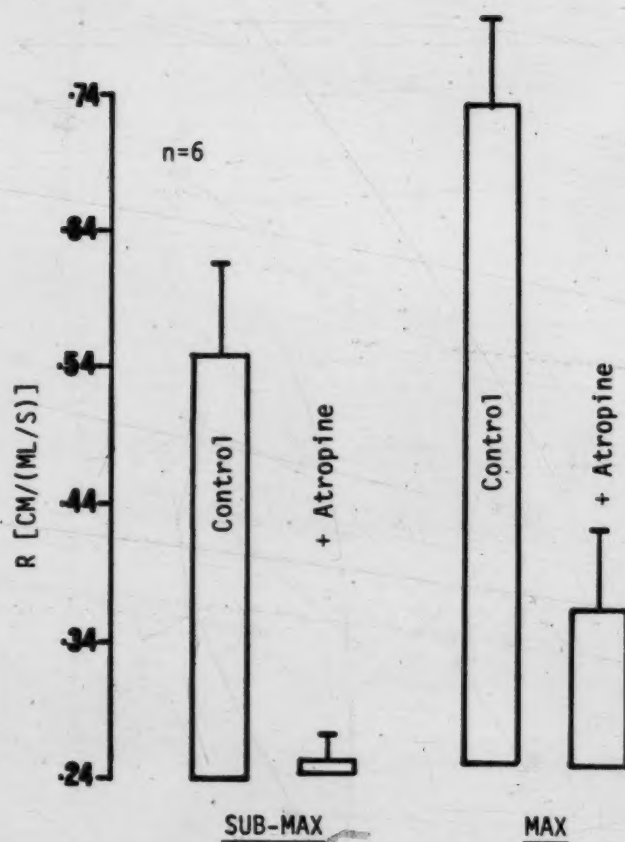


Figure 40.

The effects of atropine sulfate on absolute changes in R in response to sub-maximal (15V, 10pps, 0.5ms) and maximal (15V, 10pps, 5ms) vagal stimulation.

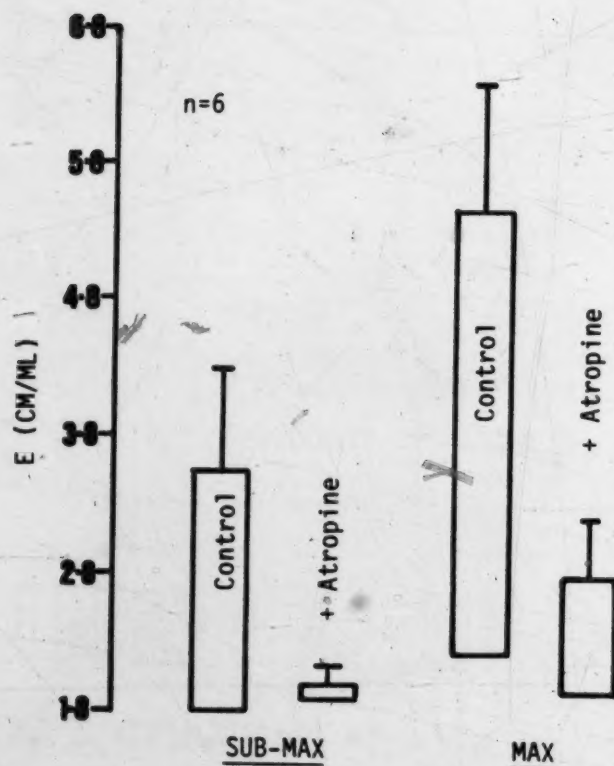


Figure 41.

The effects of atropine sulfate on absolute changes in E in response to sub-maximal (15V,10pps,0.5ms) and maximal (15V,10pps,5ms) vagal stimulation.

maximum increases in R [34.1 ± 1.5 s, $n=17$] was significantly less ($P < 0.01$) than time to maximum increases in E [43.3 ± 2.4 s, $n=17$]. When times to maximum increases in R and E were compared between controls and atropine-treated animals, the bronchoconstrictor response was significantly faster [R, $P < 0.0001$; E, $P < 0.001$] in controls than in atropine-treated animals. Therefore, after atropine treatment the late bronchoconstrictor component to nerve stimulation was observed.

4.9.3 Effects of mecamlamine

Mecamlamine (2.5 mg/kg) significantly reduced increases in R and E induced by maximal vagal stimulation (Table 24). The residual responses were further reduced, but not abolished, by atropine (Table 24). As in atropine-treated animals, the times to peak increases in R [26.8 ± 2.1 s, $n=7$] were significantly less ($P < 0.01$) than times to peak increases in E [48.8 ± 5.8 , $n=17$]. When times to maximal increases in R and E were compared with those of control animals, the bronchoconstrictor responses were significantly faster [R: $P < 0.001$; E: $P < 0.001$] in controls than in mecamlamine-treated animals. Therefore, like atropine, mecamlamine also revealed a delayed bronchoconstrictor component to nerve stimulation.

4.9.4 Effects of mepyramine and sodium cromoglycate

Mepyramine (0.1 mg/kg) and sodium cromoglycate (10 mg/kg) had no significant effect on increases in R and E due to vagal stimulation in either control or atropine-treated animals (Table 24).

4.9.5 Effects of morphine and naloxone

Morphine (1.0 mg/kg) significantly reduced increases in R and E after vagal stimulation in control and atropine-treated and mecamlamine-treated animals (Table 25). Morphine's inhibitory action was not reversed by naloxone in doses of 10 μ g/kg. Naloxone (0.2 mg/kg) itself had no effect on atropine-resistant bronchoconstriction induced by vagal stimulation (Fig. 42).

Table 24: Effects of autonomic blockers on nerve-stimulation-induced increases in pulmonary-flow resistance (R) and dynamic thoracic elastance (E). Values are means $\pm$ S.E.

Pretreatment mg/kg	Treatment mg/kg	n	R (% increase)		E (% increase)	
			Control	Test	Control	Test
—	Mecamylamine 2.5	4	233.2 $\pm$ 47.6	64.8 $\pm$ 27.9†	187.2 $\pm$ 39.2	53.6 $\pm$ 16.9†
Mecamylamine 2.5	Atropine 0.5	4	64.8 $\pm$ 27.9	24.4 $\pm$ 7.9	53.6 $\pm$ 16.9	21.4 $\pm$ 11.2
—	Mepyramine 0.1	6	101.8 $\pm$ 26.1	86.2 $\pm$ 29.3	153.6 $\pm$ 47.4	139.6 $\pm$ 31.3
Atropine 0.5	Mepyramine 0.1	6	66.8 $\pm$ 17.3	47.8 $\pm$ 19.5	36.5 $\pm$ 14.9	21.8 $\pm$ 8.9
—	Sodium cromoglycate 10	4	214.8 $\pm$ 16.5	212.7 $\pm$ 14.0	254.3 $\pm$ 32.5	215.3 $\pm$ 28.2
Atropine 0.5	Sodium cromoglycate 10	4	66.4 $\pm$ 11.3	59.6 $\pm$ 8.7	44.2 $\pm$ 11.9	33.8 $\pm$ 7.1

† P = <0.05

Table 25: Effects of nerve stimulation before and after morphine (1.0 mg/kg), in animals pretreated and untreated with drugs. The results are shown as percentage increase in pulmonary-flow resistance (R) and dynamic thoracic elastance (E) from baseline. Values are means $\pm$ S.E.

Pretreatment mg/kg	n	R (% increase)		E (% increase)	
		Control	Test	Control	Test
—	6	151.5 $\pm$ 44.9	84.4 $\pm$ 40.5†	108.7 $\pm$ 32.2	58.5 $\pm$ 28.9‡
Atropine 0.5	4	89.6 $\pm$ 23.8	40.6 $\pm$ 11.8†	38.0 $\pm$ 9.9	9.8 $\pm$ 5.4‡
Mecamylamine 2.5	4	70.7 $\pm$ 15.6	29.3 $\pm$ 10.3†	38.4 $\pm$ 10.0	16.7 $\pm$ 5.9†
Capsaicin 50.0	4	145.4 $\pm$ 41.32	97.1 $\pm$ 44.6	84.1 $\pm$ 48.9	43.9 $\pm$ 22.2

† P = <0.05;

‡ P = <0.01

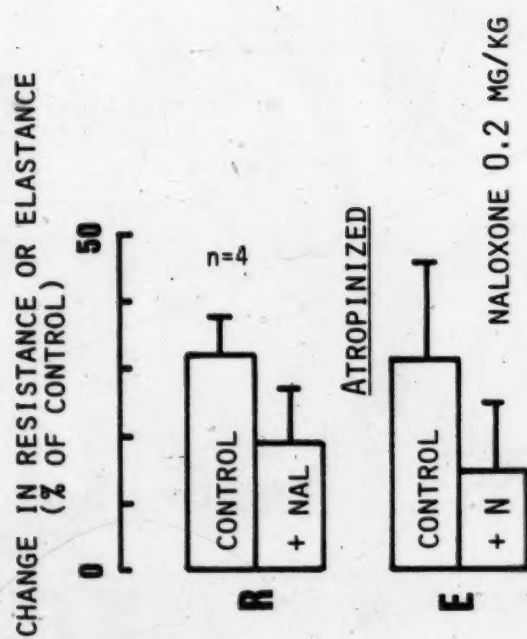


Figure 42.

The effects of naloxone on vagal stimulation induced changes in R and E.

4.9.6 Effects of capsaicin pretreatment

In capsaicin-pretreated animals, increases in R and E in response to vagal stimulation were completely abolished by acute administration of atropine (0.1 mg/kg) (Fig. 43). Morphine was without effect on airway responses to vagal stimulation in capsaicin-pretreated animals (Table 25). In these animals the times to peak bronchoconstrictor responses [R, 14.0 ± 1.4 s, $n=7$; E, 15.7 ± 0.4 s, $n=7$] were significantly less [R, $P < 0.05$; E, $P < 0.001$] than those of untreated controls [R, 16.8 ± 0.8 s, $n=17$; E, 22.2 ± 0.6 s, $n=17$]. Therefore, capsaicin-pretreatment abolished the delayed bronchoconstrictor component without affecting the atropine-sensitive early response. Times to peak increases in R and E were similar in capsaicin-pretreated animals.

4.9.7 Effects of antagonists to substance P

[D-Pro²,D-Trp^{7,9}]-SP (1.0 mg/kg) significantly reduced increases in R but not in E (Fig. 44). Higher doses of this antagonist (2.0 mg/kg) even converted increases in R to decreases, i.e., bronchodilation in the large airways ensued.

Infusion of [D-Arg¹,D-Pro²,D-Trp^{7,9},Leu¹¹]-SP significantly reduced increases in R and E induced by vagal stimulation in atropine-treated animals (Fig. 45). Unlike other antagonist, [D-Arg¹,D-Pro²,D-Trp^{7,9},Leu¹¹]-SP did not reverse the effects of vagal stimulation in large airways.

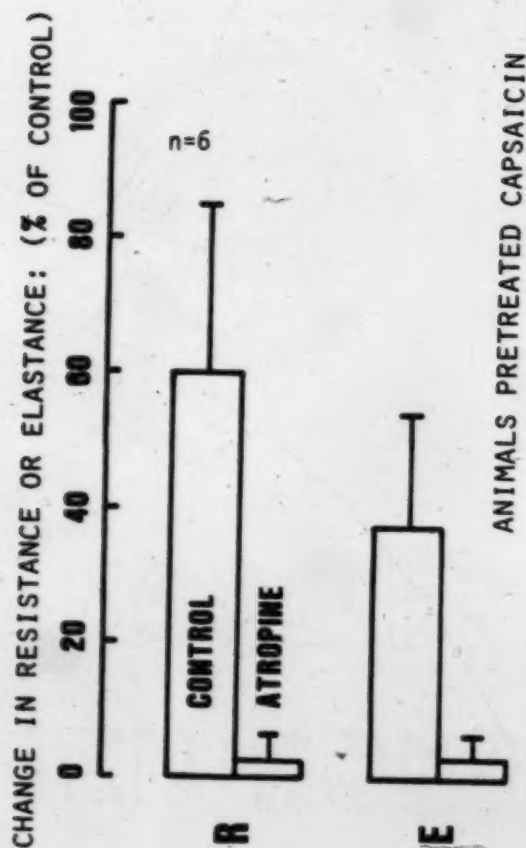


Figure 43.

The effects of atropine sulfate (0.1 mg/kg) on increases in R and E induced by vagal stimulation in capsaicin-pretreated animals.

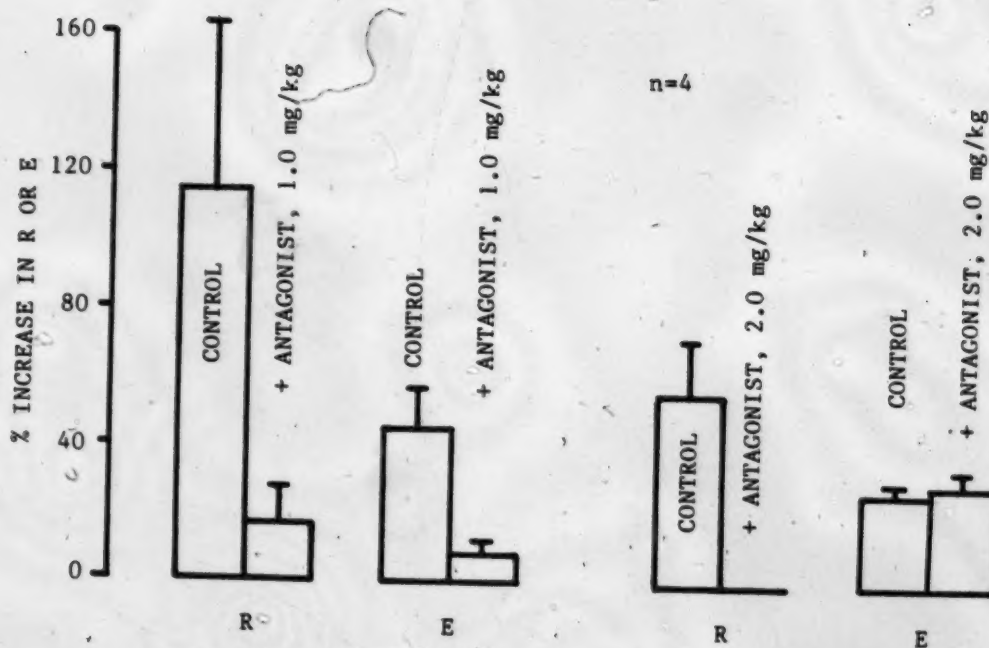


Figure 44.

The effects of [D-Pro³,D-Trp⁷,⁷]-SP on atropine-resistant increases in R and E in response to vagal stimulation.

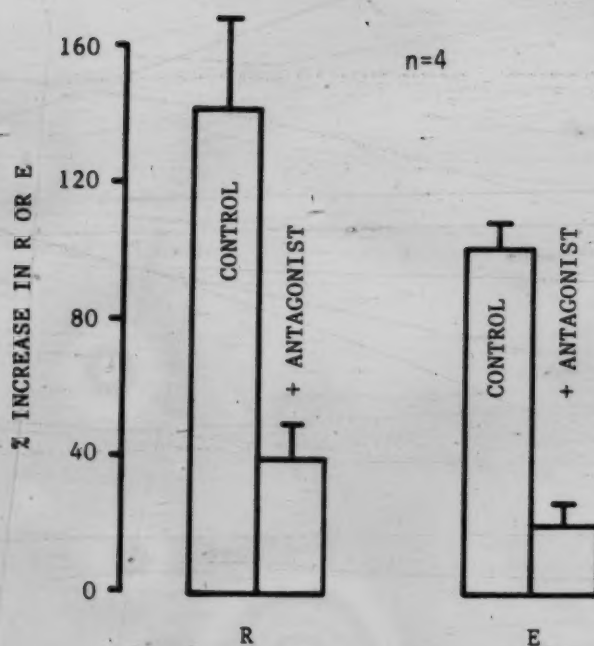


Figure 45.

The effects of [D-Arg¹,D-Pro³,D-Trp^{7,7},Leu¹¹]-SP (1.0+0.3 mg/kg) on atropine-resistant increases in R and E in response to vagal stimulation.

5. DISCUSSION

The aim of this study was to determine whether agonists can be used to selectively stimulate groups of afferent C-fiber receptors mediating reflex bronchoconstriction. The agonists used were: phenylbiguanide, lobeline, nicotine, serotonin, and capsaicin (see Paintal, 1973; Ginzel, 1975; Coleridge & Coleridge, 1984). The results with phenylbiguanide were suggestive of chemoreceptor stimulation so a comparison was made with the classical chemoreceptor stimulant, sodium cyanide (Comroe & Mortimer, 1964; McQueen, 1980). Similarly, responses to nicotine and lobeline were compared with those to DMPP, a highly selective ganglionic stimulant (Taylor, 1985).

Phenylbiguanide failed to induce bronchospasm in guinea pigs, whether it was injected into the right side of the heart (and presumably reaches pulmonary J receptors in high concentration), or intra-aortically. This is different from its effects in other species, in which it induces reflex bronchospasm (Paintal, 1977). Thus, in guinea pigs, none of the receptor groups stimulated by phenylbiguanide appears to activate reflex bronchoconstrictor pathways. However, phenylbiguanide had marked effects on MABP. It induced a dose-dependent increase after injection into the superior vena cava or right atrium, and a dose-dependent transient fall followed by a longer rise in MABP after i.a. injection. The drug had insignificant effects on heart rate — an unexpected finding considering the accepted view of normal homeostatic control mechanisms and the size of the changes in MABP. These changes were solely reflex in origin. They were absent in animals in which the brain had been destroyed, were reduced by bilateral vagotomy or glossopharyngealotomy, and were almost eliminated by combining these nerve sections and by mecamylamine. Responses to phenylbiguanide were mediated via the sympathetic nervous system because pretreatment with 6-hydroxydopamine prevented responses, and bethanidine, an adrenergic neurone-blocking agent, reduced both pressor and depressor responses. Interestingly, atropine reduced the pressor but not the depressor response. As bilateral vagotomy reduced both pressor and depressor responses, our findings suggest that atropine acts by blocking the effects of phenylbiguanide on afferent receptors, and that it is highly unlikely to block the sympathetic

efferent pathway. The depressor response is mediated via vagal rather than glossopharyngeal afferents, as it was abolished by bilateral vagotomy and was unaffected by bilateral glossopharyngealotomy.

High concentrations of phenylbiguanide stimulate not only J receptors but also chemoreceptors (Paintal, 1977; Anand & Paintal, 1980). Thus in our experiments, phenylbiguanide could possibly stimulate the unmyelinated afferents of arterial chemo- and (possibly) baro-receptors (Paintal, 1973; Ginzel, 1975; Kirchheim, 1976; Coleridge & Coleridge, 1984, 1985). The effects of phenylbiguanide on MABP we observed are the algebraic sum of the pressor (positive) and depressor (negative) responses occurring simultaneously/sequentially due to stimulation of a variety of receptors. Chemoreceptor stimulation causes reflex hypertension (Comroe & Mortimer, 1964; Eyzaguirre & Zapata, 1984), thus, it is likely that phenylbiguanide's hypertensive effects are due to stimulation of chemoreceptors. Chemoreceptors located in the pulmonary circulation and the heart, as well as those located in the aortic arch and carotid bodies could be involved. Hypertensive responses to phenylbiguanide are similar after either i.v. or i.a. injection suggesting that it is unlikely that chemoreceptors located in heart or pulmonary circulation play a significant role, however, in the absence of experiments in which nerve fibers were sectioned selectively this is not certain. Comroe & Mortimer (1964) suggest that the hypertensive responses to phenylbiguanide result from stimulation of aortic arch chemoreceptors rather than carotid body chemoreceptors which mediate hypotension. In our experiments, the hypertensive responses were mediated by both aortic-arch- and carotid-body-chemoreceptors, as both bilateral vagotomy and bilateral glossopharyngealotomy reduced the responses. This conclusion is supported by our results with sodium cyanide, which induced reflex hypertension after injection i.v. or i.a.

The prompt depressor response following injection of phenylbiguanide i.a. and its absence after the drug was injected i.v., implies that the depressor response arises from stimulation of the receptor-rich sensory areas in the aortic arch or coronary circulation (Bezold-Jarish reflex) (Paintal, 1973) via coronary and/or aortic baroreceptors with unmyelinated afferents (Anand & Paintal, 1980). This conclusion is supported by the

reduction of the depressor response following bilateral vagotomy, and the lack of effect of bilateral glossopharyngealotomy on the depressor response. There is a large population of baroreceptors with unmyelinated afferents (Rees, 1967), and, interestingly, relatively large doses of phenylbiguanide are necessary to produce a depressor response. This is probably because simultaneous chemoreceptor stimulation causes hypertensive responses, and masks the depressor responses. This could explain the absence of a depressor response after injection of phenylbiguanide i.v. Here phenylbiguanide encounters pulmonary and cardiac chemoreceptors mediating pressor responses before reaching baroreceptors mediating the depressor response. Secondly, the absence of a depressor response after injection i.v. could be due to dilution of the phenylbiguanide during passage through the pulmonary circulation, to concentrations that are ineffective or weakly effective in stimulating unmyelinated afferents and the resulting effect could easily be masked by the larger hypertensive responses due to chemoreceptor stimulation.

Sodium cyanide, a specific stimulant of chemoreceptors (Comroe & Mortimer, 1964; Jain et al., 1972; McQueen, 1980) had no bronchoconstrictor effect in guinea-pig airways. Like phenylbiguanide, sodium cyanide injected i.v. or i.a., induced reflex hypertensive responses as evidenced by results with nerve sections. These results could be attributed to chemoreceptor stimulation. Absence of any hypotensive effect after i.a. injection of sodium cyanide, suggests that phenylbiguanide's depressor responses are unlikely to be due to chemoreceptor stimulation.

Like phenylbiguanide, lobeline, in doses at which it is known to be a selective stimulant of J receptors in cat and man (Jain et al., 1972), had no bronchospastic effects in guinea pigs, irrespective of its route of administration, and, in the doses used, it had no significant effects on MABP. This lack of effect on MABP could be due to balancing out of a number of opposing influences, e.g., excitation of sympathetic and parasympathetic ganglia, excitation of aortic and carotid chemoreceptors which in turn will induce compensatory cardiovascular reflexes. Higher doses of lobeline were not used because they would no longer be selective for afferent endings (Ginzel, 1975). Moreover, our doses were more than

sufficient to stimulate afferent receptors in cats (Bevan & Verity, 1961), rabbits (Hara & Kikuta, 1958), and man (Stern et al., 1966; Jain et al., 1972).

By contrast, nicotine, an afferent receptor and ganglionic stimulant (Ginzel, 1975), induced pressor responses rather than depressor responses forming part of a 'pulmonary chemoreflex' arising from J receptors (Dawes & Comroe, 1954; Paintal, 1973). These hypertensive responses were not due to release of catecholamines from the sympathetic nerve endings (Taylor, 1985) as similar responses were present in animals pretreated with reserpine, an agent that depletes catecholamine stores in sympathetic nerve terminals. Hypertensive responses were non-reflex. Bilateral vagotomy and mecamlamine were without effect, thus excluding a role for afferent receptor-induced reflex changes in MABP. It is possible that nicotine's pressor effect results from release of catecholamines from the adrenal medulla, though the finding that mecamlamine did not reduce the pressor response does not support this suggestion. The hypertensive responses could be due to stimulation of sympathetic ganglia, and are unlikely to be due to paralysis of parasympathetic ganglia. This conclusion is supported by the hypertensive responses seen after injection of DMPP, a selective ganglionic stimulant (Taylor, 1985), i.v. and i.a. Finally, nicotine had no bronchoconstrictor effects in the doses used.

Unlike the agonists used before, 5-H.T. induced dose-dependent increases in R and E. The increases in R were greater after i.v. than after injection of the same dose i.a., suggesting that 5-H.T. acts preferentially on receptors accessible by i.v. injection. It is unlikely that 5-H.T. exerts its effects after passage through systemic circulation, as injection of drug into the dorsal aorta (2 expts.) did not induce bronchospasm. Thus, the presence of bronchospasm following injection of 5-H.T. i.a. indicates that the receptors sensitive to 5-H.T. are accessible via the bronchial circulation. These receptors could be located in the bronchial circulation itself, or may be located in the pulmonary circulation and accessed via bronchopulmonary anastomoses (Willis, 1919/20; Verloop, 1949). Regardless of the route of administration, 5-H.T. caused non-reflex bronchospasm — the ganglionic blockers mecamlamine, pentolinium, and trimetaphan; the muscarinic blocker atropine, and the

adrenergic neurone blocker bethanidine, and bilateral vagotomy were without effect on the increases in R and E. Thus, 5-H.T.-induced bronchospasm is not caused by stimulation of sensory receptors forming part of a reflex bronchospastic pathway, but is due solely to a direct effect on bronchial smooth muscle. The absence of 5-H.T.-induced bronchospasm after methysergide, a 5-H.T. antagonist (Douglas, 1985), confirms that 5-H.T.-induced bronchospasm results from a direct effect on bronchial smooth muscle. It is not possible to suggest the receptor type (5-H.T.₁ or 5-H.T.₂) involved as selective antagonists were not used.

Capsaicin induced bronchospasm whether given i.v. or i.a. In low doses ($<8.0 \mu\text{g/kg}$) it induced dose-dependent increases in R and E and in higher doses it caused tachyphylaxis. The drug's effects on both R and E indicate that it acts on both conducting and distensible airways in guinea pigs (Nadel, 1965). After capsaicin i.v., responses were always greater than with the same doses given i.a.; when responses of about equal size were compared, the only difference was a longer time to maximal response in R after i.a. administration. It is thus apparent that the bronchial (systemic) circulation contains capsaicin-sensitive neurons that are stimulated when the drug is given i.a. It is unlikely that capsaicin reaching the lungs after i.a. injection and passage through the systemic arterial and venous systems induces bronchospasm, as injection of this drug into the dorsal aorta had no significant bronchospastic effect. Capsaicin reaching the pulmonary circulation via anastomoses into the bronchial circulation (Willis, 1919/1920; Verloop, 1949) probably accounts for the increases in E after injection i.a. Interestingly, the ratio of the percentage change from baseline for R and E was similar whether the drug was given i.v. or i.a., and the dose that induced tachyphylaxis was similar for the two routes.

Capsaicin appears to induce non-reflex bronchospasm in guinea pigs, in marked contrast to its effects in other species (Karczewski & Widdicombe, 1969b; Paintal, 1969; Russell & Laai-Fook, 1979; Dixon et al., 1980). Responses to capsaicin, i.v. or i.a., were similar in decentralized animals and in intact controls, and neither bilateral vagotomy nor bilateral glossopharyngealotomy altered capsaicin-induced responses. Moreover, the

ganglion-blocker mecamylamine, the muscarinic blocker atropine, and the adrenergic neuron-blocker bethanidine failed to modify capsaicin-induced changes in R and E. Thus, in guinea pigs, capsaicin-induced bronchospasm has no autonomic reflex component, whether the drug is given i.v. or into receptor-rich areas of the systemic circulation. These findings are in general agreement with those of Molnar et al., (1969) who injected capsaicin into the external jugular vein in guinea pigs.

The choice of anesthetic can modify reflex responses to afferent nerve stimulation and to drugs (e.g., Neil et al., 1949a, 1949b). We found that increases in R and E in response to the same doses of capsaicin (i.v.) were similar in animals anesthetized with urethane, pentobarbital, or α -chloralose. Also, neither mecamylamine nor atropine altered capsaicin-induced responses nor revealed a reflex component in these animals.

Phenylbiguanide injected i.v. induces hypertension, and when injected i.a., it induces hypotension followed by hypertension. All these changes in MABP are mediated by a central reflex arc. These changes are unlike those caused by J receptor stimulation because: 1. reflex hypotension as a component of the 'pulmonary chemoreflex' (Dawes & Comroe, 1954) is observed following J receptor stimulation, and reflex hypertension was the dominant finding in our experiments; 2. reflex hypotension was observed after injection of phenylbiguanide i.a. rather than i.v. which would not have been the case if J receptors were involved (Paintal, 1969); 3. hypertensive responses were similar after i.v. or i.a. injections of phenylbiguanide; thus it is unlikely that these effects are mediated via J receptors. Clearly, phenylbiguanide's effects on MABP are not mediated via J receptor stimulation unlike responses seen in cats, rats, and rabbits (see Coleridge & Coleridge, 1984). Other stimulants of afferent C-fibers, e.g., lobeline, nicotine, and 5-H.T., given i.v., also failed to induce depressor responses attributable to J receptor stimulation. Thus, lobeline had no effects on MABP, and nicotine induced pressor responses of a 'non-reflex' nature. Capsaicin given i.v. or i.a. did cause falls in MABP, but like this drug's bronchospastic effects, the falls were not reflexly mediated. Thus, J receptors appear to have few roles in guinea pigs, in contrast to their effects in cats, dogs, rats, rabbits, and humans (see Paintal, 1973; Coleridge & Coleridge, 1984).

Phenylbiguanide is bronchospastic in cats and rabbits (Karczewski & Widdicombe, 1969b; Paintal, 1969; Anand & Paintal, 1980) but not in dogs or humans (Coleridge & Coleridge, 1984) and, according to the present study, lacks any bronchospastic effects in guinea pigs. Similarly, lobeline and nicotine, in the doses used, had no bronchospastic effects, and 5-H.T. only induced bronchospasm via a direct effect on bronchial smooth muscle. Also, capsaicin induced non-reflex bronchospasm in guinea pigs, in contrast to the drug's effects in dogs (Russel & Lai-Fook, 1979), cats (Coleridge & Coleridge, 1984), and rats (Sapru et al., 1981). In dog, cat, and rat capsaicin induces reflex bronchospasm by stimulating C-fiber afferents. These findings taken together suggest that the innervation of guinea-pig lung may differ from that of other species, including man. Thus, guinea-pig lung may contain few, if any, sensory neurons forming part of a reflex bronchospastic pathway. It is also possible that guinea pigs' lungs contain afferent C-fibers that are insensitive to the chemical stimulants we used. Species differences in the sensitivity of J receptors to different chemicals are known (see Table 6), but, it seems unlikely that guinea pigs could have significant numbers of functional J receptors so resistant to such a wide variety of chemicals. Therefore, it appears that guinea pigs' lungs contain minimal numbers of the typical J receptors described by Paintal (1969) that form part of the reflex bronchospastic arc.

At this stage one can only speculate as to why guinea pigs' lungs are devoid of J receptors. According to Paintal (1970) the normal physiologic stimulus for J receptor stimulation in man is pulmonary congestion, which in man, under physiological conditions, occurs during exercise especially at high altitudes. Pulmonary congestion can also occur in pathological conditions, e.g., left ventricular failure. This J receptor stimulation leads to reflex inhibition of the muscles of the leg (J reflex) (see Fig. 7), thereby inhibiting exercise, which, in turn, activates the J reflex by causing pulmonary congestion. The absence of J receptors in guinea pigs, could be the result of an adaptation to altitude in their country of origin, the high plains of Peru (Wagner, 1976).

The H_1 -receptor antagonist mepyramine blocked the depressor response to phenylbiguanide. Similarly, H_1 -receptor blockers eliminate active reflex vasodilation in

response to carotid-sinus-nerve stimulation in dogs (Boerth, 1972). Mepyramine could have blocked phenylbiguanide's effects on sensory receptors, as H_1 -receptor antagonists in general block the stimulatory effects of histamine on pulmonary sensory receptors (Paintal, 1977; Sampson & Vidruk, 1979), or, mepyramine could have blocked the effects of histamine released by reflex stimulation of efferent nerves. The present experiments did not permit delineation of the specific groups of sensory receptors responsible for, or contributing to, the responses observed.

In guinea pigs, it has been shown that ASA, tartrazine, and diazoxide induce bronchospasm by reflex mechanisms involving H_1 -receptors, and that this effect is blocked by SCG (Biggs, 1984). In guinea pigs, phenylbiguanide has no bronchospastic effects but shows reflex effects on blood pressure. The latter are blocked by both mepyramine and SCG. Thus, there is evidence that SCG may block the neurogenic release of histamine from cells at sites other than in the lungs and activated via another neural pathway. Applying these results to the findings by Shanahan and his co-workers (1984) of various types of "mast" cells, it appears that selective stimulation of different afferent (and presumably efferent) pathways could induce the neurogenic release of histamine from different types of these cells. None of these findings preclude actions of SCG on both afferent receptors and efferent pathways: a mechanism that could account for effects which seem paradoxical.

Capsaicin induced non-reflex bronchospasm, which was unaffected by pretreatment with mepyramine. It has been suggested that capsaicin induces its effects by releasing substance P (discussed below) (Jessel et al., 1978; Buck & Burke, 1983), found in sensory nerve endings in guinea pigs and several other mammalian species (see Pernow, 1983), but not in human lung (Laitinen et al., 1983). SCG had no effect on the bronchospastic actions of capsaicin in our experiments in guinea pigs, though it is said to reduce capsaicin-induced vagal afferent activity in dogs (Dixon et al., 1980).

Guinea-pig lungs receive sensory innervation containing SP or SP-like immunoreactive material (Nilsson et al., 1977; Wharton et al., 1979; Lundberg et al., 1984), and systemic treatment with capsaicin in this species results in almost complete loss of SP-like

immunoreactivity from pulmonary sensory neurons (Lundberg et al., 1983a, 1983b, 1984; Hua et al., 1985). This hypothesis suggests that capsaicin induces bronchospasm via release of SP or related tachykinins from vagal afferents. In our experiments with animals injected with capsaicin (50 mg/kg, s.c.) 7 days earlier, capsaicin given i.v. or i.a. in doses up to 30 times greater than threshold failed to increase R and E. To test whether capsaicin's effects are due to the release of SP or related tachykinins, we first analyzed the effects of selected tachykinins on guinea-pig airways in vivo.

SP and other related tachykinins like PH and ERP induced dose-dependent increases in R and E, whether given i.v. or i.a., and the order of their potency was the same by either route. Relative potencies (SP=1.0) were PH=4.9, ERP=1.7 for R and PH=5.1, ERP=1.8 for E, indicating ERP as less potent than PH. In preliminary experiments, ERP was approximately 3X less potent than eledoisin. If this is taken into consideration, eledoisin's potency relative to SP is 5.1 and 5.4 for R and E, values that accord with those of others (Hua et al., 1984; Karlsson et al., 1984). None of the tachykinins was significantly more effective in increasing R than E, indicating a similar distribution of SP-P and SP-E receptors in the large (conducting) and small distensible airways. As with capsaicin, increases in both R and E were invariably larger in response to i.v. than to i.a. injection. However, the times to peak response for each tachykinin were similar by either route, and injection of the tachykinins into the dorsal aorta failed to induce bronchospasm. Thus, the tachykinins' effects after both i.v. and i.a. injection arise from the 'first pass' of the drugs through the pulmonary and bronchial/pulmonary circulations. R and E returned to baseline values within 5 min of induction of the responses, indicating that plasma extravasation, if it occurs (Hua et al., 1984; Lundberg et al., 1984) does not alter baseline values of R and E at the dose levels of the tachykinins used. Infusion of the tachykinins for 30 min caused sustained increases in R and E, with no evidence of tachyphylaxis, and responses to single injections at the end of the infusion were not significantly different from those recorded before infusion. These findings show that airway smooth muscle does not develop tachyphylaxis to the effects of these tachykinins (Karlsson et al., 1984), unlike ileal smooth muscle (Lee et al., 1982).

Increases in R and E induced by all three tachykinins appear to result from a direct action on airway smooth muscle. No reflex component of the response could be identified — atropine, mecamlamine, and bilateral vagotomy were without effect.

Lundberg et al., (1984) has proposed that bronchospastic tachykinins are released from unmyelinated afferent endings in response to injections of capsaicin, or nerve stimulation. The unmyelinated afferents from the lungs are carried in the vagus and the sympathetic nerves of spinal origin (see Coleridge & Coleridge, 1984). Therefore, tachykinins could be released from both vagal (Lundberg et al., 1984) and spinal afferents (Saria et al., 1985). To test whether these bronchospastic tachykinins are released from vagal afferents we performed experiments in which the vagi were stimulated.

Our findings show that peripherally directed uni- or bi-lateral vagal stimulation increases R and E in agreement with findings in other species (Colebatch & Halmagyi, 1963; Olsen et al., 1965; Karczewski & Widdicombe, 1969a). Unilateral stimulation caused only 20-30% of the increase in R in response to bilateral stimulation probably due to shift of ventilation away from the lung of stimulated side. This phenomenon of shift in ventilation is called *pendelluft* (Otis et al., 1956). This buffering action is less prominent in the peripheral airways as changes in E after bilateral stimulation were only twice that after unilateral stimulation. This could be due to the larger tidal volume used (5-7 ml compared with 2.5 ml in spontaneously breathing animals), thereby keeping the alveoli distended and giving little scope for compensation. Central stimulation of one cut vagus with the other intact had no bronchospastic effects, indicating minimal or zero vagal crossover in the brain stem. Any feeble activity resulting via the contralateral vagus would be nullified by the buffering action mentioned above.

The rapid rates of increase in R and E, and their prompt reversibility suggest that they are due to contraction of smooth muscle rather than mucosal edema or obstruction of the lumens of the airways by mucus. Olsen et al., (1965) suggested that increases in R represented contraction of bronchial smooth muscle, while the increases in E represented reduction in the number of distensible units resulting from contraction of the smooth muscle

in the alveolar duct region. In our experiments, the increases in E were longer lasting than those in R. Increases in R occur due to contraction of smooth muscle in large airways, and thus the effects should be rapidly reversible. Increases in E occur due to contraction of smooth muscle in peripheral airways, including smooth muscle in alveolar duct region, thus partly or completely blocking the air entry into the alveoli. When air entry is partly blocked the alveoli become overinflated because of the phenomenon of "flow limitation" (Pedley et al., 1977), as discussed in Section 1.9.2. When the alveoli are overinflated the elasticity of the lungs is increased (Strieder, 1976). On the other hand when air entry is completely blocked, the alveoli collapse. These collapsed alveoli apparently take a longer time to reexpand, thus accounting for the longer lasting effect on E as compared to an effect on R. In such a situation, one would also expect an increase in R, because the same volume of air is being pushed into a reduced volume lung. However, we did not observe persistent increases in R. This could be due in part to simultaneous dilation occurring in the large airways. These possibilities were not explored.

The bronchospasm observed following nerve stimulation had two distinct components: 1) an early response due to release of acetylcholine from vagal *efferents* and blocked by the muscarinic blocker, atropine, and the ganglion blocker, mecamylamine (Colebatch & Halmagyi, 1963; Olsen et al., 1965; Karczewski & Widdicombe, 1969a; Woolcock et al., 1969; Nadel, 1980a; Fryer & MacLagan, 1984), 2) a delayed response, due to the release of capsaicin-sensitive peptide hormones from vagal *afferents*. This effect is absent in capsaicin-pretreated animals (Lundberg et al., 1983a, 1983b; Martling et al., 1984), and is unaffected by ganglion blockers and atropine. The delayed response was absent when the vagi were stimulated submaximally indicating that it is stimulus-strength dependent. This could explain why some workers failed to observe the delayed response (Nadel, 1980a; Fryer & MacLagan, 1984).

Bronchoconstrictor responses to vagal nerve stimulation (discussed later) were unaffected by mepyramine and SCG — results similar to those seen with capsaicin. After pretreatment with atropine, vagal stimulation induced smaller responses which were again

unaffected by SCG. Thus, SCG has no blocking action on vagal efferents, and does not prevent the release of SP from afferent nerve endings as a result of antidromic stimulation of vagal afferents (Lundberg et al., 1983a, 1983b). This is in good agreement with the lack of effect of SCG on capsaicin-induced bronchospasm.

The recent development of synthetic SP-analogs with antagonist properties (Folkers et al., 1981; Rosell & Folkers, 1982) has provided tools to study the roles of SP in physiologic processes. Most of the work with these antagonists have been done *in vitro*. Studies in intact animals are lacking. We investigated the actions of [D-Pro²,D-Trp^{7,8}]-SP (Holmdahl et al., 1981; Leander et al., 1981; Hakanson et al., 1982; Karlsson et al., 1984; Leander et al., 1984) and [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP against three tachykinins (SP, ERP, and PH), to establish antagonist specificities. Subsequently, these antagonists were used to help analyze the bronchoconstrictor responses to capsaicin and to nerve stimulation after atropine treatment.

SP- and PH-induced increases in R and E were unchanged after i.v. injection of [D-Pro²,D-Trp^{7,8}]-SP (1.0-2.0 mg/kg). Our findings with [D-Pro²,D-Trp^{7,8}]-SP on SP and PH were obtained in guinea pig airways *in vivo*, whereas much of the evidence identifying this agent as an antagonist to SP was obtained on airway smooth muscle *in vitro* (Karlsson & Persson, 1983; Karlsson et al., 1984), where high concentrations were required to inhibit SP-induced contractions, e.g., 100 μ M in the case of guinea-pig hilar bronchi. Thus, the dose of antagonist used in our experiments (2 mg/kg) could have been insufficient to block SP- and PH-induced increases in R and E. By contrast, ERP-induced increases in R and E were reduced by [D-Pro²,D-Trp^{7,8}]-SP. Blockade of ERP-induced bronchospasm by this antagonist suggests that different receptor sites mediate responses to ERP as compared to PH and SP. Since ERP is a selective agonist at the SP-E receptor (Brown & Hill, 1983), it implies that [D-Pro²,D-Trp^{7,8}]-SP is a better antagonist at SP-E than at SP-P receptors. Studies by others (Briggs et al., 1983; Briggs & Jamieson, 1983) *in vitro* support our findings *in vivo* that [D-Pro²,D-Trp^{7,8}]-SP is selective at SP-E receptors.

[D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP reduced SP-, and PH-induced increases in R and E, without affecting ERP-induced increases in either parameter, supporting the concept of

multiple tachykinin receptors. SP and PH are more selective at SP-P receptors (Brown & Hill, 1983), thus [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP has greater affinity for SP-P receptors, a finding supported by other's experiments in vitro (Mandahl & Bill, 1983).

Our findings with the two SP-antagonists suggest that there are fundamental differences in their effects on the airways. Although the specificity of currently available SP-antagonists has been questioned (see Karlsson et al., 1984; Post et al., 1985), neither of the antagonists examined affected responses to 5-H.T. Therefore, the differences in specificity observed appear to reflect selective inhibition among the various tachykinins.

[D-Pro²,D-Trp^{7,8}]-SP (1.0 mg/kg), was without effect on capsaicin-induced increases in R and E, but increasing the dose of antagonist to 2.0 mg/kg reduced capsaicin-induced increases in R. This SP-antagonist reduced increases in R induced by nerve stimulation in animals pretreated with atropine. In fact, in both cases, flow resistance decreased, therefore bronchodilation ensued in (presumably) the large airways. This bronchodilator response was seen despite the fact that capsaicin and nerve stimulation still induced increases in E in the presence of the antagonist. The selective reversal of capsaicin- and nerve-stimulation-induced increases in R without affecting increases in E suggests that bronchodilator transmitters may be released in large airways in addition to bronchoconstrictor tachykinins. In animals in which SP has been depleted from afferent nerve terminals by pretreatment with capsaicin, the bronchodilator responses to capsaicin and nerve stimulation were absent after atropine. This suggests that capsaicin may deplete an inhibitory transmitter released in response to vagal stimulation. One possible candidate is VIP — but this was not explored further.

Lundberg et al., (1983c) reported that [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP abolished airway responses to capsaicin, SP, and vagal stimulation in guinea pigs in vivo: findings similar to ours. However, in their experiments, drug-induced bronchospasm was estimated from measurements of peak intratracheal pressure by the method of Konzett & Rossler (1940): a technique that cannot distinguish effects on the conducting and distensible airways. We found that [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP reduced nerve stimulation-induced increases in R and E. Similarly, it reduced capsaicin-induced increases in R but had no effect

on increases in E. Increases in R due to capsaicin or nerve stimulation were not abolished by [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP. About 25-30% of increases in R due to capsaicin, and about 15-20% of increases in R due to nerve stimulation were still present after [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP. These residual responses could be due to possibly insufficient amounts of antagonist, or due to a lack of specificity of the antagonist against the transmitters released by capsaicin or nerve stimulation. Unlike, our findings with [D-Pro²,D-Trp^{7,8}]-SP, we observed no bronchodilation in large or small airways after capsaicin or nerve stimulation. The lack of bronchodilator response could be attributed to : 1. the presence of a small residual bronchoconstriction after [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP; or 2. non-specific effects of [D-Arg¹,D-Pro²,D-Trp^{7,8},Leu¹¹]-SP on the inhibitory transmitters. Our present studies cannot distinguish between these two possibilities.

Assuming the specificities of the two antagonists, some deductions can be made from our findings with capsaicin- and nerve-stimulation-induced increases in R and E. In the large airways tachykinins, acting on SP-P and SP-E receptors, are released by capsaicin or antidromic stimulation of vagal afferents, though tachykinins which are more effective at SP-E receptors predominate. In peripheral airways, the majority of the tachykinins released act at SP-P receptors. Our findings confirm the report that electrical stimulation releases different mediators in various generations of bronchi in guinea-pigs' lung (Grundstrom et al., 1981).

In vitro SP contracts intestinal smooth muscle both directly, and indirectly via the release of acetylcholine (Holzer & Lembeck, 1980; Holzer et al., 1981). Cholinergic antagonists also reduce, but never abolish, intestinal contractions to capsaicin (Bartho & Szolcsanyi, 1978; Bartho et al., 1982). Similarly, atropine reduces the responses by rabbit airways to SP both in vitro and in vivo (Tanaka & Grunstein, 1984; Grunstein et al., 1984): effects which are unchanged by ganglionic blockade or by tetrodotoxin. The lack of effect of atropine on capsaicin-induced responses in our animal model shows that, at least in guinea-pig lung, neither the tachykinins nor capsaicin act indirectly via the release of acetylcholine.

Fewtrell et al., (1982) working with rat peritoneal mast cells, demonstrated that a variety of tachykinins release histamine. They and others (Foreman, 1983; Brown & Hill, 1983) contend that the histamine-releasing properties of tachykinins are related to their basic N-terminal residues (see Fig. 8). Thus, SP is much more potent than ERP in releasing histamine, and PH lacks this property. The lack of effect of mepyramine on the bronchospasm induced by SP, PH, and ERP, shows that in guinea-pig airways in vivo, these tachykinins (in the doses tested) do not release histamine. Gater et al., (1982) showed that the dose of SP required to release histamine is 1000 times greater than that required to contract the smooth muscle of the guinea-pig ileum. However, the lack of effect of mepyramine on capsaicin- or nerve-stimulation-induced (atropine-resistant) bronchospasm rules out any release of histamine, especially when one considers the exquisite sensitivity of guinea-pig airways to histamine.

It has been suggested that exogenous opioids modulate intestinal responses to capsaicin; presumably by reducing drug-induced release of SP (Bartho et al., 1982). In our experiments, morphine reduced capsaicin-induced increases in R and E. Similarly, morphine also reduced airway responses to nerve stimulation after animals were injected with atropine or mecamlamine. However, in animals pretreated with capsaicin, morphine had no effect on airway responses to nerve stimulation, which suggests a role of morphine in inhibiting the release of tachykinins. The inhibitory effect of morphine was not reversed by naloxone, implying that this effect is not an action of morphine on μ receptors. Furthermore, as naloxone itself neither enhanced nor inhibited responses to capsaicin, endogenous opioids acting via μ receptors can play little (if any) part in capsaicin-induced airway responses in guinea pigs.

Morphine also reduced PH-induced increases in R and E but had no effect on airway responses to SP or ERP. It is tempting to speculate that PH, like capsaicin, may act partly by releasing SP or other tachykinins. If PH was indeed acting via release of SP, then the depletion of SP from afferent nerve endings by capsaicin pretreatment should reduce airway responses due to PH but not due to SP or ERP. Treatment of animals with capsaicin

(50 mg/kg, s.c.) 7 days earlier, had no effect on any of the tachykinin-induced increases in R. However, SP- and PH-induced increases in E were reduced in treated animals as compared to controls, though increases E due to ERP were not affected. It seems unlikely that PH releases SP or other tachykinin in the lungs because: 1. capsaicin pretreatment only reduced PH-induced increases in E, but had no effect on increases in R; 2. SP-induced increases in E were also reduced along with PH-induced increases in E. The possible mechanism for the PH- and SP-induced reduction in E could possibly include reduction in the number of SP-P receptors or increased metabolism of tachykinins.

Potential animal models of asthma are of two broad types: those which develop spontaneous episodes of bronchoconstriction, and those in which bronchoconstriction is produced by experimental conditions, such as challenge with antigen, pharmacologic agents or inhaled irritants. Basenji-Greyhound dogs are a good example of the first type of animal model (Hirshman et al., 1980), and the guinea pig is considered to be a good model of the second type (see Section 2.4). In our experiments, the lack of reflex bronchospastic responses to various chemicals used, suggests that innervation of guinea-pigs' lung may differ from that of other species, including man. In guinea pigs, there may be few, if any, sensory neurons forming part of a bronchospastic reflex arc. It is not necessary to suggest from the above findings that the guinea pig lacks usefulness as an animal model for studying the pathophysiology of asthma. Asthma occurs as a result of the interaction of a variety of influences (see Fig. 11) and it is unlikely that any single animal species will resemble man in all aspects of asthma. Therefore, the studies reported in this thesis suggest that, although the guinea pig may not be a good model to study the reflex bronchospastic effects (especially those from pulmonary receptors), it is a good model to study the role of tachykinins in lung diseases.

In conclusion, the findings reported in this thesis show that none of the afferent C-fiber stimulants used, induce reflex bronchospasm in guinea pigs, despite their ability to do so in other species, e.g., cats, dogs, rabbits, and rats. Whichever group (or groups) of neurons is affected by these agonists, none forms part of a sensory pathway that is involved

in a centrally mediated bronchospastic reflex arc. Phenylbiguanide induced reflex changes in MABP, probably by acting on aortic and carotid chemoreceptors. Capsaicin induced non-reflex bronchospasm, probably by releasing SP from the endings of primary afferent neurons that are accessible from both the pulmonary and bronchial circulations. [D-Pro², D-Trp^{7,8}]-SP is more specific at SP-E receptors, whereas [D-Arg¹, D-Pro², D-Trp^{7,8}, Leu¹¹]-SP is more specific at SP-P receptors. Based on the specificity of the SP-antagonists in our experiments, it appears that in the large airways SP-E receptor specific tachykinins are mainly released, but in the small airways SP-P receptor specific tachykinins predominate.

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7. APPENDIX

1. Program for baseline adjustment.

```

5 DIM V%(1)
10 DISPLAYCLEAR
15 H$='DC Voltage measurement using preamp channels A & B'
20 HTEXT('UNDERLINE',1,15,H$)
25 MOVECURSOR(3,1)
30 PRINT 'Ensure that both channel A and B preamp controls are set for';
35 PRINT 'voltage' \ PRINT 'measurement (V) and programmable gain (P)'
40 FOR I=8 TO 9
45 TESTGAIN(I,A,A$)
50 IF A<0 GO TO 55 \ IF A$='V' GO TO 70
55 HTEXT('FLASH',6,1,'Check for bad preamp setting on channel A')
60 MOVECURSOR(6,41) \ IF I=9 THEN PRINT 'B'
65 PAUSE(2) \ GO TO 45
70 NEXT I
75 SETGAIN( ,2,8,2)
80 HTEXT('FLASH',6,1,'Enter an M to make a measurement or a Q to quit')
85 MOVECURSOR(9,1) \ GETCHAR(Q$) \ IF LEN(Q$)=0 GO TO 85
90 ERASETEXT( ,9)
95 IF Q$='Q' GO TO 115 \ IF Q$<>'M' GO TO 80 \ AIN( ,V%( ),2, ,8,2)
100 MOVECURSOR(8,1) \ PRINT 'Preamp channel A measures ';
105 PRINT USING '#####. # mv and channel B #####. # mV',V%(0)/2,V%(1)/2
110 GO TO 80
115 ERASETEXT( ,6)
120 END

```

2. Program for calibration.

```

10 DIM M$(57) \ DIM F%(499)
15 FOR I=1 TO 57 \ READ M$(I) \ NEXT I
20 DATA '*** PULMONARY MEASURING SYSTEM - CALIBRATION ROUTINE ***'
25 DATA 'Preamp channels A & B must be set to measure voltage (V)'
30 DATA 'and for programmable gain (P).'
35 DATA 'Set preamp'
40 DATA 'for programmable gain (P)'
45 DATA 'for voltage measurement (V)'
50 DATA 'Pressure calibration'
55 DATA 'Apply atmospheric pressure to the transducer & connect it to'
60 DATA 'MINC preamp A through GRASS channel 1. Select BRIDGE 2K input'
65 DATA '& balance the system in the usual way. Press the VT105 space'
70 DATA 'bar when you are ready to proceed.'
75 DATA 'Adjust the GRASS baseline controls to obtain an input level'
80 DATA 'of less than 50 mV.'
85 DATA 'crease the GRASS baseline control & press the VT105 space bar'
90 DATA 'Apply some test pressure to the transducer just larger than'
95 DATA 'the maximum you will need and adjust the GRASS sensitivity'
100 DATA 'and CAL controls for a suitable downward pen deflection.'
105 DATA 'Enter the pressure in m of water'
110 DATA 'change the polarity of the pressure signal and try again.'
115 DATA '#####. # m of water corresponds to #####. # % full scale.'

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```

120 DATA 'crease the GRASS sensitivity or CAL control'
125 DATA 'Baseline of ####.# mV and ####.# mV per cm of water'
130 DATA 'Volume calibration'
135 DATA 'Zero adjustment'
140 DATA 'MIN preamp B through GRASS channel 2. Selet BRIDGE 2K input'
145 DATA 'Input level is ####.# mV'
150 DATA 'Baseline level is ####.# mV'
155 DATA 'Adjust the GRASS sensitivity & CAL controls so that a '
160 DATA 'suitable downward flow rate pen deflection is obtained for'
165 DATA 'the desired volume and pump speed.'
170 DATA 'Enter the volume in millilitres'
175 DATA 'Collecting data for 10 seconds'
180 DATA 'Change the polarity of the flow rate signal & try again'
185 DATA 'Peak flow rate is ####.# % of full scale'
190 DATA 'Bad input data - Try again'
195 DATA 'Baseline of ####.# % of full scale'
200 DATA 'Press Y if the flow rate waveform is ok , N if not - '
205 DATA 'Calibration printout'
210 DATA 'Turn the LA120 on and prepare it for the calibration report'
215 DATA 'GRASS channel 1 (the pressure transducer)'
220 DATA 'Enter the '
225 DATA 'Balance voltage switch setting '
230 DATA 'Balance + or - setting '
235 DATA 'Balance dial reading '
240 DATA 'Sensitivity setting '
245 DATA 'CAL setting '
250 DATA 'Polarity + or - setting '
255 DATA 'Amp HI FREQ setting '
260 DATA 'Baseline setting (NB from -5 to +5 !)'
265 DATA '*** PULMONARY MEASURING SYSTEM - CALIBRATION REPORT ***'
270 DATA 'Balance polarity '
275 DATA 'Polarity '
280 DATA 'GRASS Channel 2 (the flow rate transducer)'
285 DATA '.....'
290 DATA 'Enter pump speed in rpm '
295 DATA 'Calibration volume ##.# ml'
300 DATA 'Pump speed ####.# rpm'
305 DATA 'DISPLAYCLEAR \ HTEXT('UNDERLINE',1,12,M$(1)) \
MOVECURSOR(3,1)
310 PRINT M$(2) \ PRINT M$(3) \ C$='A'
315 FOR I=0 TO 1
320 TESTGAIN(8+I,A,A$) \ IF I=1 THEN C$='B'
325 MOVECURSOR(6,1)
330 IF A<0 THEN PRINT M$(4);C$;M$(5) \ PAUSE(2) \ GO TO 320
335 IF A$<>'V' THEN PRINT M$(4);C$;M$(6) \ PAUSE(2) \ GO TO 320
340 NEXT I
345 SETGAIN( ,2,8,2)
350 ERASETEXT( ,6) \ HTEXT('FLASH',6,1,M$(7)) \ MOVECURSOR(8,1)
355 FOR I=8 TO 11 \ HTEXT('FLASH',6,1,M$(7)) \ MOVECURSOR(13,1)
360 GETCHAR(Q$) \ IF LEN(Q$)=0 GO TO 360
365 MOVECURSOR(13,1) \ PRINT M$(24) \ PRINT \ PRINT M$(12)
370 PRINT M$(13) \ T1=2*50
375 MOVECURSOR(18,1) \ AIN( ,D%, ,8)
380 PRINT USING M$(26),D%/2
385 IF ABS(D%)<=T1 THEN 405
390 IF D%<-T1 THEN PRINT 'In'+M$(14)

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```

395 IF D%>T1 THEN PRINT 'De'+M$(14)
400 GETCHAR(Q$) \ ON LEN(Q$)+1 GO TO 400,375
405 FOR I=14 TO 19 \ ERASETEXT(,I) \ NEXT I
410 MOVECURSOR(15,1) \ PRINT USING M$(27),D%/2
415 HTEXT(,17,1,'Pressure measurement') \ MOVECURSOR(19,1)
420 FOR I=15 TO 17 \ PRINT M$(I) \ NEXT I
425 MOVECURSOR(23,1) \ PRINT M$(18); \ INPUT X \ IF X<=0 GO TO 425
430 FOR I=18 TO 24 \ ERASETEXT(,I) \ NEXT I \ MOVECURSOR(19,1)
435 AIN(,Y%,,8) \ IF Y%<0 THEN PRINT M$(19) \ PAUSE(3) \ GO TO 305
440 MOVECURSOR(19,1) \ P=100*Y%/2047 \ PRINT USING M$(20),X,P
445 IF Y%<2047 THEN IF Y%>=1500 THEN 465
450 IF Y%>=2047 THEN PRINT 'De'+M$(21)
455 IF Y%<1500 THEN PRINT 'In'+M$(21)
460 MOVECURSOR(22,1) \ PRINT M$(18); \ INPUT X \ IF X<=0 GO TO 460 \ GO TO
435
465 PAUSE(3) \ DISPLAYCLEAR \ HTEXT('UNDERLINE',1,1,M$(1))
470 MOVECURSOR(22,1) \ PRINT M$(7) \ PRINT
475 PRINT USING M$(22),D%/2,(Y%-D%)/(2*X)
480 HTEXT('FLASH',7,1,M$(23)) \ MOVECURSOR(9,1)
485 PRINT M$(8) \ PRINT M$(25) \ PRINT M$(10) \ PRINT M$(11) \
MOVECURSOR(14,1)
490 GETCHAR(Q$) \ IF LEN(Q$)=0 GO TO 490
495 MOVE CURSOR(14,1) \ PRINT M$(24) \ PRINT \ PRINT M$(12) \ PRINT M$(13)
500 MOVECURSOR(19,1) \ AIN(,Z%,,9) \ PRINT USING M$(26),Z%/2
505 IF ABS(Z%)<=T1 THEN 525
510 IF Z%>T1 THEN PRINT "De'+M$(14)
515 IF Z%<-T1 THEN PRINT 'In'+M$(14)
520 GETCHAR(Q$) \ ON LEN(Q$)+1 GO TO 520,500
525 FOR I=15 TO 23 \ ERASETEXT(,I) \ NEXT I
530 MOVECURSOR(16,1) \ PRINT USING M$(27),Z%/2
535 PRINT \ PRINT 'Volume measurement' \ PRINT
540 FOR I=28 TO 30 \ PRINT M$(I) \ NEXT I
545 MOVECURSOR(23,1) \ PRINT M$(31); \ INPUT V \ IF V<=0 GO TO 545
550 HTEXT('FLASH',24,40,M$(32))
555 AIN(,F%(,500,1/50,9) \ M1=0 \ M2=0
560 FOR I=0 TO 499 \ IF F%(I)>M1 THEN M1=F%(I)
565 IF F%(I)<M2 THEN M2=F%(I)
570 NEXT I
575 FOR I=19 TO 24 \ ERASETEXT(,I) \ NEXT I \ MOVECURSOR(20,1)
580 IF M1<=0 GO TO 585 \ IF ABS(M2)<M1 GO TO 590
585 PRINT M$(33) \ PAUSE(3) \ GO TO 465
590 P=100*M1/2047 \ PRINT USING M$(34),P
595 IF M1<2047 THEN IF M1>1500 THEN 615
600 IF M1>=2047 THEN PRINT 'De'+M$(21)
605 IF M1<1500 THEN PRINT 'In'+M$(21)
610 MOVECURSOR(22,1) \ PRINT M$(31); \ INPUT V \ IF V<=0 GO TO 610 \ GO TO
550
615 T2=Z%+.1*(M1-Z%) \ T3=Z%+.25*(M1-Z%) \ I=-1
620 I=I+1 \ IF F%(I)>T2 THEN IF I>=499 GO TO 675 \ GO TO 620
625 I=I-1
630 I=I+1 \ IF I>499 GO TO 675 \ IF F%(I)<T3 THEN 630
635 I1=I \ I=I-1 \ S=0
640 I=I+1 \ IF I>499 GO TO 675 \ IF F%(I)>T3 THEN S=S+F%(I)-Z% \ GO TO 640
645 I=I-1 \ I2=1
650 I2=I2+1 \ IF I2>499 GO TO 675
655 IF F%(I2)-Z%>S/100 THEN S=S+F%(I2)-Z% \ GO TO 650

```



```

660 I1=I1-1 \ IF I1<0 GO TO 675
665 IF F%(I1)-Z%>S/100 THEN S=S+F%(I1)-Z% \ GO TO 660
670 IF I2-I1<400 GO TO 680
675 ERASETEXT(,24) \ HTEXT('FLASH',24,1,M$(35)) \ GO TO 535
680 I2=I2-1 \ I1=I1+1 \ K=2000*V/(S/50)
685 DISPLAYCLEAR
690 REGION('FULL',1)
695 GRAPH('LINES,SHADE,-UNITS',I2-I1+1,,F%(I1),,Z%,1,)
700 MOVECURSOR(21,1) \ PRINT USING M$(36),Z%/2,K \ PRINT \ PRINT M$(37)
704 MOVECURSOR(23,54)
705 GETCHAR(Q$) \ IF LEN(Q$)=0 GO TO 705
710 DISPLAYCLEAR \ IF Q$='N' GO TO 525 \ IF Q$<>'Y' GO TO 705
715 HTEXT('UNDERLINE',1,1,M$(1))
720 HTEXT('FLASH',3,1,M$(38)) \ MOVECURSOR(5,1) \ PRINT M$(39) \ PRINT
725 PRINT M$(40) \ OPEN 'LP;' FOR OUTPUT AS FILE 1
730 GOSUB 1000
735 PRINT #1 \ PRINT #1,M$(50);' ' ;CLK$;' ' ;DAT$ \ PRINT #1
740 PRINT #1,TAB(10)M$(54) \ PRINT #1 \ PRINT #1,TAB(10)M$(40) \ GOSUB 2000
745 PRINT #1 \ PRINT #1 \ PRINT #1,TAB(10);
750 PRINT #1,USING M$(22),D%/2,(Y%-D%)/(2*X) \ PRINT #1
755 PRINT #1,TAB(10)M$(54) \ PRINT #1
760 FOR I=7 TO 24 \ ERASETEXT(,I) \ NEXT I
765 MOVECURSOR(7,1) \ PRINT M$(53) \ GOSUB 1000
770 PRINT M$(55); \ INPUT C7 \ PRINT #1,TAB(10)M$(53) \ GOSUB 2000
775 PRINT #1,TAB(15) \ PRINT #1,USING M$(56),V \ PRINT #1,TAB(15);
780 PRINT #1,USING M$(57),C7 \ PRINT #1 \ PRINT #1
785 PRINT #1,TAB(10); \ PRINT #1,USING M$(36),Z%/2,K
790 PRINT #1 \ PRINT #1,TAB(10)M$(54)
795 FOR I=1 TO 54 \ PRINT #1 \ NEXT I
800 CLOSE #1 \ DISPLAYCLEAR \ HTEXT('UNDERLINE',8,8,M$(1))
805 MOVECURSOR(12,1) \ PRINT M$(7) \ PRINT
810 PRINT USING M$(22),D%/2,(Y%-D%)/(2*X)
815 MOVECURSOR(17,1) \ PRINT M$(23) \ PRINT
820 PRINT USING M$(36),Z%/2,K
825 MOVECURSOR(20,1) \ GO TO 3000
1000 MOVECURSOR(9,1) \ PRINT M$(41)+M$(42); INPUT C1
1005 PRINT M$(41)+M$(43) \ MOVECURSOR(10,34)
1015 GETCHAR(A1$) \ IF A1$='+' GO TO 1020 \ IF A1$<>'-' GO TO 1015
1020 PRINT \ PRINT M$(41)+M$(44); \ INPUT C2
1025 PRINT M$(41)+M$(45); \ INPUT C3
1030 PRINT M$(41)+M$(46); \ INPUT C4
1035 PRINT M$(41)+M$(47) \ MOVECURSOR(14,35)
1045 GETCHAR(A2$) \ IF A2$='+' GO TO 1050 \ IF A2$<>'-' GO TO 1045
1050 PRINT \ PRINT M$(41)+M$(48); \ INPUT C5
1055 PRINT M$(41)+M$(49); \ INPUT C6
1060 RETURN
2000 PRINT #1 \ PRINT #1,TAB(15); \ PRINT #1,USING M$(42)+'###',C1
2005 PRINT #1,TAB(15)M$(51)+'(' +A1$+')'
2010 PRINT #1,TAB(15); \ PRINT #1,USING M$(44)+'###.###',C2
2015 PRINT #1,TAB(15); \ PRINT #1,USING M$(45)+'###.###',C3
2020 PRINT #1,TAB(15); \ PRINT #1,USING M$(46)+'###',C4
2025 PRINT #1,TAB(15)M$(52)+'(' +A2$+')'
2030 PRINT #1,TAB(15); \ PRINT #1,USING M$(48)+'###',C5
2035 PRINT #1,TAB(15); \ PRINT #1,USING 'Baseline setting ###',C6
2040 RETURN
3000 END

```

3. Program for calculation of R and E.

```

10 PRINT 'The Pulmonary Data Collection program will begin soon.'
15 DIM #1,G%(29999) \ DIM F%(1499),R(200),C(200),T(200)
20 DIM #4,M$(49)=80
25 OPEN 'VM:PM.DAT' AS FILE 1
30 OPEN 'PMS005.CHR' FOR INPUT AS FILE 3
35 OPEN 'VM:PM.CHR' AS FILE 4
40 FOR I=1 TO 49 \ INPUT #3,M$(I) \ NEXT I \ CLOSE 3
45 DISPLAYCLEAR \ HTEXT('U',1,12,M$(1))
50 MOVECURSOR(3,1) \ PRINT M$(2) \ PRINT M$(3) \ C$='A' \ FOR I=0 TO 1
55 TESTGAIN(8+I,A,A$) \ IF I=1 THEN C$='B'
60 MOVECURSOR(6,1) \ IF A<0 THEN PRINT M$(4);C$;M$(5) \ PAUSE(2) \ GO TO 55
65 IF A$<>'V' THEN PRINT M$(4);C$;M$(6) \ PAUSE(2) \ GO TO 55
70 NEXT I \ SETGAIN(2,8,2) \ ERASETEXT(,6) \ MOVECURSOR(6,1)
75 FOR I=7 TO 10 \ PRINT M$(I) \ NEXT I
80 FOR I=0 TO 1 \ MOVECURSOR(11+3*I,1) \ PRINT USING 'Channel #',I+1
85 AIN(,Z2%,...,*+1) \ MOVECURSOR(12+3*I,1) \ PRINT USING M$(11),Z2%/2
90 IF ABS(Z2%)<100 GO TO 115
95 IF Z2%>=100 THEN PRINT 'De'M$(12)
100 IF Z2%<=-100 THEN PRINT 'In'+M$(12)
105 MOVECURSOR(13+3*I,70)
110 GETCHAR(Q$) \ ON LEN(Q$)+1 GO TO 110,85
115 IF I=0 THEN Z1%=Z2%
120 ERASETEXT(,13+3*I) \ NEXT I \ FOR I=2 TO 16 \ ERASETEXT(,I) \ NEXT I
125 MOVECURSOR(3,1) \ PRINT USING M$(13),Z1%/2,Z2%/2
130 ERASETEXT(,5) \ MOVECURSOR(5,1) \ PRINT M$(14); \ INPUT K1
135 IF K1<=0 GO TO 130 \ K1=2*K1
140 ERASETEXT(,6) \ MOVECURSOR(6,1) \ PRINT M$(15); \ INPUT K2
145 IF K2<=0 GO TO 140 \ K2=K2/2000
150 DISPLAYCLEAR \ HTEXT('UNDERLINE',1,12,M$(1))
155 MOVECURSOR(3,1) \ PRINT USING M$(13),Z1%/2,Z2%/2
160 MOVECURSOR(5,1) \ PRINT USING M$(33),K1/2 \ PRINT USING M$(34),K2*2000
165 ERASETEXT(,8) \ MOVECURSOR(8,1) \ PRINT M$(16); \ INPUT D1$
170 J=0 \ IF LEN(D1$)>6 GO TO 165 \ IF LEN(D1$)=0 GO TO 165
175 FOR I=1 TO LEN(D1$) \ K=ASC(SEG$(D1$,I,1)) \ IF K>90 THEN J=1
180 IF K<48 THEN J=1
185 IF I=1 THEN IF K<65 THEN J=1
190 IF K<65 THEN IF K>57 THEN J=1
195 NEXT I \ IF J=1 GO TO 165
200 OPEN D1$ FOR OUTPUT AS FILE 2
205 MOVECURSOR(10,1) \ PRINT M$(17) \ INPUT D2$
210 IF LEN(D2$)>80 THEN ERASETEXT(,11) \ GO TO 205
215 AIN('DISPLAY',I1%,...8)
220 HTEXT('FLASH',21,1,M$(18)) \ MOVECURSOR(23,1) \ PRINT M$(19);
225 GETCHAR(Q$) \ IF LEN(Q$)<1 GO TO 225
230 IF Q$<>'G' GO TO 225 \ ERASETEXT(,23)
235 HTEXT('FLASH',23,20,M$(20)) \ TIS=CLK$
240 AIN('DISPLAY',F%( ),1500,1/50,8,2) \ DISPLAYCLEAR
245 HTEXT('UNDERLINE',1,12,M$(1)) \ MOVECURSOR(3,1)
250 PRINT M$(21) \ N1%=1499 \ I2%=1 \ J%=0 \ M1=0 \ M2=0
255 FOR I=0 TO 1499 \ G%(I)=F%(I) \ NEXT I
260 MOVECURSOR(5,1) \ PRINT M$(22) \ PRINT M$(23)

```

```

265 GOSUB 2000 \ IF I2% >= 1499 GO TO 280 \ IF I1% < 1 GO TO 265
270 M1=M1+R(J%) \ M2=M2+C(J%)
275 PRINT USING M$(24),J%,R(J%),C(J%),T(J%) \ GO TO 265
280 PRINT \ M1=M1/J% \ M2=M2/J% \ PRINT USING M$(25),M1,M2
285 PRINT \ FOR I=26 TO 28 \ PRINT M$(I) \ NEXT I
290 GETCHAR(Q$) \ IF LEN(Q$) < 1 GO TO 290
295 IF Q$='Q' GO TO 520 \ IF Q$='R' GO TO 215 \ IF Q$ <> 'C' GO TO 290
300 J1%=J% \ DISPLAYCLEAR \ HTEXT('UNDERLINE',1,12,M$(1)) \
MOVECURSOR(3,1)
305 PRINT #2,M$(30)+D1$+M$(31)+DAT$ \ PRINT #2
310 PRINT #2,D2$ \ PRINT #2 \ PRINT #2,USING M$(33),K1/2
315 PRINT #2,USING M$(34),K2*2000 \ PRINT #2
320 PRINT #2,USING M$(32)+T1$,J1% \ PRINT #2
325 PRINT #2,M$(22) \ PRINT #2,M$(23)
330 FOR I=1 TO J% \ PRINT #2,USING M$(24),I,R(I),C(I),T(I) \ NEXT I
335 PRINT #2 \ PRINT #2,USING M$(25),M1,M2 \ PRINT #2 \ PRINT M$(29)
340 GETCHAR(Q$) \ IF LEN(Q$) < 1 GO TO 340
345 IF Q$ <> 'S' GO TO 340
350 J%=0 \ T2$=CLK$ \ AIN('DISPLAY,CONTINUOUS',F%( ),1500,1/50,8,2)
355 HTEXT('FLASH',21,1,M$(35)) \ MOVECURSOR(22,1) \ PRINT TAB(15)M$(36)
360 PRINT TAB(19)M$(37) \ PRINT TAB(12)M$(38); \ MOVECURSOR(23,73)
365 WAITFOR DATA(F%( ),I%)
370 FOR K%=0% TO 749% \ G%(J%+K%)=F%(K%+I%) \ NEXT K% \ J%=J%+750%
375 GETCHAR(Q$) \ IF LEN(Q$) > 0 GO TO 385
380 IF J% < 30000 GO TO 365
385 TERMINATE('IMMEDIATE'F%( )) \ IF Q$='T' GO TO 395
390 IF LEN(Q$) > 0 GO TO 520
395 DISPLAYCLEAR \ HTEXT('UNDERLINE',1,12,M$(1)) \ MOVECURSOR(3,1)
400 PRINT M$(39) \ N1%=J%-1 \ I2%=1 \ J%=0
405 MOVECURSOR(6,1) \ PRINT M$(47) \ PRINT M$(48)
410 MOVECURSOR(8,1) \ GOSUB 2000 \ IF I1% < 1 GO TO 420
412 R2=100*R(J%)/M1 \ C2=100*C(J%)/M2
415 PRINT USING M$(49),J%,R(J%),R2,C(J%),C2,T(J%)
420 IF I2% < N1% GO TO 410
425 FOR I=1 TO 8 \ ERASETEXT(I) \ NEXT I
430 MOVECURSOR(3,1) \ PRINT M$(40) \ J2%=J%
435 PRINT #2,USING M$(41)+T2$,J2% \ PRINT #2 \ PRINT #2,M$(47)
440 PRINT #2,M$(48)
442 FOR I=1 TO J2% \ R2=100*R(I)/M1 \ C2=100*C(I)/M2
445 PRINT #2,USING M$(49),I,R(I),R2,C(I),C2,T(I) \ NEXT I
450 CLOSE 2 \ FOR I=3 TO 8 \ ERASETEXT(I) \ NEXT I
455 MOVECURSOR(3,1) \ PRINT M$(42) \ PRINT M$(43)
460 GETCHAR(Q$) \ IF LEN(Q$) < 1 GO TO 460
465 IF Q$='N' GO TO 485 \ IF Q$ <> 'Y' GO TO 460
470 OPEN 'LP:' FOR OUTPUT AS FILE 3 \ OPEN D1$ FOR INPUT AS FILE 2
475 LINPUT #2,L$ \ PRINT #3,L$ \ IF END #2 THEN 480 \ GO TO 475
480 CLOSE 2 \ CLOSE 3
485 REGION('UPPER',1) \ REGION('LOWER',2)
490 GRAPH('LINES,SHADE',J2%,T(1),R(1),,M1,1,)
495 GRAPH('LINES,SHADE',J2%,T(1),C(1),,M2,2,)
500 LABEL(,,'Resistance',1) \ LABEL(,,'Elastance',2)
505 MOVECURSOR(21,1) \ PRINT M$(44) \ PRINT M$(45) \ PRINT ' ' + M$(28);
510 GETCHAR(Q$) \ IF LEN(Q$) < 1 GO TO 510
515 IF Q$='C' GO TO 150 \ IF Q$='R' GO TO 45 \ IF Q$ <> 'Q' GO TO 510
520 CLOSE 1 \ KILL 'VM:PM.DAT' \ PRINT M$(46)
525 CLOSE 4 \ KILL 'VM:PM.CHR' \ GO TO 9000

```



```

2000 I1%=I2% \ S=0 \ S1=0 \ S2=0 \ S3=0 \ S4=0 \ S5=0 \ S6=0
2005 IF I1%>=N1% THEN 2070 \ IF G%(I1%)>205 THEN I1%=I1%+2 \ GO TO 2005
2010 IF I1%>=N1% THEN 2070 \ IF G%(I1%)<307 THEN I1%=I1%+2 \ GO TO 2010
2015 I2%=I1%-2
2020 I2%=I2%+2 \ IF I2%>N1% GO TO 2070
2025 IF G%(I2%)-Z2%>S/100 THEN S=S+G%(I2%)-Z2% \ GO TO 2020
2030 I1%=I1%-2 \ IF I1%<1 THEN 2065
2035 IF G%(I1%)-Z2%>S/100 THEN S=S+G%(I1%)-Z2% \ GO TO 2030
2040 I1%=I1%+2 \ I2%=I2%-2 \ IF I2%-I1%<40 THEN I1%=0 \ GO TO 2065
2041 J%=J%+1 \ T(J%)=(I1%-1)/100
2045 FOR I%=I1% TO I2% STEP 2 \ X1=(G%(I%-1)-Z%)/K1 \ X2=K2*(G%(I%)-Z2%)
2050 S1=S1+X2/50 \ S2=S2+X1*S1 \ S3=S3+X1*X2 \ S4=S4+S1*S1
2055 S5=S5+X2*S1 \ S6=S6+X2*X2 \ NEXT I%
2060 R(J%)=(S4*S3-S2*S5)/(S4*S6-S5*S5) \ C(J%)=S4/(S2-R(J%)*S5)
2065 C(J%)=1/C(J%) \ I2%=I2%+20 \ RETURN
2070 I1%=N1% \ I2%=N1% \ RETURN
9000 END

```

*** PULMONARY MEASURING SYSTEM - DATA COLLECTION ***

Preamp channels A & B must be set to measure voltage (V)
and for programmable gain (P).

Set preamp

for programmable gain (P)

for voltage measurement (V)

Use GRASS channel 1 for the pressure transducer and channel 2
for flow rate. Apply atmospheric pressure to both transducers
and adjust the balance controls in normal way. Then adjust
the baseline controls for an input level of less than 50 mV.

Input level is #####.## mV

increase the GRASS baseline control & press the VT105 space bar

channel A baseline is #####.## mV, channel B is #####.## mV

Input pressure calibration in mV per cm of water

Input flow rate calibration in mls per volt second

Enter a valid data file name

Enter up to one line of text to identify the data file

Control measurements

When you press G control data will be collected for 15 secs

Collecting data for 15 seconds

Wait while the control data are processed

	Resistance cm/(ml/s)	Elastance cm/ml	Elapsed Time seconds
###	#####	#####	#####
Mean	#####	#####	

Enter an R if you wish to repeat the control measurements

a C if you wish to continue with the experiment

a Q to quit

Enter an S when you are ready to start collecting more data

*** PULMONARY MEASURING SYSTEM *** FILE - .DAT

I Control measurements (##)

started -

Pressure calibration of #####.## mV per cm of water

Volume calibration of ##### millilitres per volt second

Experimental data

Enter an A if you wish to abort the experiment

or a T if you wish to terminate it prematurely

(You may have to wait up to 15 secs before action is taken)

Wait while the experimental data is processed

Calculations complete - filing the results

II Experimental measurements (###) started -

If you want to print the data file prepare the LA120

and enter a Y, if not enter an N

Enter a C if you wish to continue with new control data

an R to restart the complete experiment

Program terminated

Resistance			Elastance		Elapsed Time	
	cm/(ml/s)	%	cm/ml	%		seconds
###	###.###	###.##	###.###	###.##		###.##